Severe respiratory syncytial virus (RSV) disease requiring intensive care in children

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Thesis submitted in accordance with the requirements of

the University of Liverpool for the degree of

Doctor of Medicine

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MBChB MMed FCPaed FRCPCH MRCP DCH

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Abstract

Introduction: Respiratory syncytial virus (RSV) is the most important viral cause for lower respiratory infection and respiratory failure leading to paediatric intensive care unit (PICU) admission in infants and young children throughout the world.

Setting: 20-bed PICU in a tertiary university-affiliated, multi-disciplinary, regional referral children's hospital with an annual admission rate of approximately 1100 children.

Study patients: All children with confirmed RSV infection that required PICU admission between June 1999 and May 2009 (i.e. ten consecutive RSV seasons).

Aim: To investigate the clinical impact of severe RSV disease on children, with admission to PICU being the marker of severe RSV disease.

Results: During the ten-year study period 491 RSV-positive patients were admitted to PICU. Elevation of hepatic transaminase levels was common (46% of those studied). Disease severity as judged by duration of ventilation, inotrope use and mortality was greater in children with elevated transaminase levels. Myocardial damage or injury as indicated by elevated cardiac troponin levels were found in 35-40% of infants with structurally normal hearts and severe RSV lung disease. Significantly reduced right ventricular function was found in 20% of infants without congenital heart disease ventilated for RSV bronchiolitis, but the degree of dysfunction was not related to the levels of respiratory support or biochemical markers of inflammation.

Up to 40% of patients admitted with severe RSV bronchiolitis were infected with bacteria in their lower airways and had a longer duration of ventilation ($p<0.001$). Overall PICU mortality of RSV-positive patients was 8.6%, with a standardised mortality ratio (SMR) 0.76, PICU RSV-attributable mortality 4.4% and hospital RSV-attributable mortality 0.9%. Pre-existing disease/co-morbidity, namely congenital heart disease, chronic lung disease, large airway abnormality, immunodeficiency, neuromuscular disease (Relative Risk/RR 2.4), and in particular multiple pre-existing diseases (RR 4.4) and cardiac anomaly (RR 3.0), was associated with a significantly higher risk of death. Nosocomial RSV infection rates were: PICU-acquired 8.1% and hospital ward-acquired 7.8%. Mortality, 25% in PICU-acquired (RR 4.3) and 21% in hospital-acquired (RR 3.6), was increased, but principally by its association with pre-existing conditions/comorbidity. Nosocomial RSV infection was the strongest predictor of duration of ventilation and length of stay on PICU.

Conclusions: Myocardial and hepatocellular damage can occur in infants with severe RSV bronchiolitis with structurally normal hearts and normal ventricular function. Reduced right ventricular function occurs in infants without congenital heart disease ventilated for RSV bronchiolitis. This may explain the increased morbidity and mortality associated with RSV bronchiolitis in children with significant congenital heart disease. Pre-existing disease/co-morbidity and nosocomial infection is associated with a significantly higher risk of death from severe RSV infection.

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I would like to acknowledge and thank Dr Michael Eisenhut, co-investigator and collaborator on many studies, for his strong support, diligence and reliability, along with solid statistical advice, throughout the years it has taken for this thesis to come to fruition.

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I am especially grateful to the late Prof Tony Hart, my previous supervisor and academic mentor, for his sage perspective supplemented by steady encouragement and an understanding of balancing the demands of a fulltime clinical job with doctoral toil.

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I would like to thank Prof James Stewart, my doctoral supervisor, for stepping into the fold as supervisor at a late stage and continuing to facilitate & encourage, while sparing the whip.

* Echocardiographic measurements were performed by Dr Arjamand Shauq and Dr Shuba Narayanswamy, Dept. of Paediatric Cardiology, Royal Liverpool Children's Hospital - Alder Hey; with specific training in echocardiographic assessment of right ventricular function provided by Dr Michael Burgess, Consultant Cardiologist, University Hospital Aintree, Liverpool.

Abbreviations

RSV	respiratory syncytial virus
PICU	paediatric intensive care unit
A&E	Accident & Emergency department
HDU	high dependency unit
PIM	paediatric index of mortality
SMR	standardised mortality ratio
IQR	inter-quartile range
CI	confidence interval
SD	standard deviation
RR	relative risk
W-M-W	Wilcoxon-Mann-Whitney test
FiO_2	fraction of inspired air that is oxygen
PaO_2	partial pressure of oxygen
$PaCO_2$	partial pressure of carbon dioxide
MAP	mean airways pressure
OI	Oxygen Index
VI	Ventilation Index
CPAP	continuous positive airways pressure
HFOV	high frequency oscillatory ventilation
ECLS	extracorporeal life support
ECMO	extracorporeal membrane oxygenation
ECG	electrocardiography

CHD	congenital heart disease
URTI	upper respiratory tract infections
LRTI	lower respiratory tract infections
BAL	broncho-alveolar lavage
CFU	colony forming units
CRP	C-reactive protein
IFN	interferon
IL	interleukin
C	chemokine
TNF	tumour necrosis factor
TREM-1	triggering receptor expressed on myeloid cells – type 1
RSVIG	RSV immunoglobulin
cTnT	cardiac troponin T
ALT	alanine transaminase
AST	asparate transaminase

Dedication

Firstly, I would like to dedicate this MD thesis to the critically ill children studied, and their families.

Secondly, I would like to dedicate this MD thesis to my wife, Karen, for her ceaseless support & belief, and my children: the two, Christiane & Benedikt, old enough to suffer from doctoral-induced neglect and the youngest, Dominik, too young to allow such neglect.

Lastly, I would also like to dedicate this MD thesis to the late Prof Tony Hart, sage, supervisor, mentor, and the reason for my doctoral affiliation with The University of Liverpool.

Declaration

This thesis is the result of my own work. The material contained in the thesis has not been presented, nor is currently being presented, either wholly or in part for any other degree or other qualification.

The work was carried out in the Paediatric Intensive Care Unit, and investigations in the Departments of Medical Microbiology & Clinical Biochemistry, of the Royal Liverpool Children's Hospital - Alder Hey.

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1 Introduction

1.1 *Respiratory Syncytial Virus (RSV)*

Although outbreaks of pyrexial respiratory illnesses have been described for many centuries, probably the first description of an outbreak of respiratory syncytial virus (RSV) was reported 1941 (Adams 1941). Adams described an outbreak of nosocomial chest infections in thirty-two infants in a neonatal unit that resulted in 9 deaths, with cytoplasmic inclusions identified in the lungs at autopsy. RSV was first identified in 1956 from a colony of chimpanzees with coryza and designated "chimpanzee coryzal agent" (Blount, Morris and Savage 1956). Subsequently a year later in 1957 it was isolated from children with lung disease in Baltimore, USA (Chanock and Finberg 1957; Chanock, Roizman and Myers 1957). Since then RSV has been recognised as the single most important virus causing acute respiratory tract infections in children (Howard et al. 2000; Hall 2001; Crowe, Collins and Knipe 2007; Hall 2009).

Because of its characteristic cytopathologic findings in tissue culture where RSV-infected epithelial cells clump together forming syncytia (giant multinucleated cells), the virus was named respiratory syncytial virus, as suggested by Chanock and colleagues (Chanock et al. 1961; Chanock et al. 1962). Syncytium formation has been found to be both an *in vitro* and an *in vivo* phenomenon.

Respiratory syncytial virus (RSV) is the most important viral cause for lower respiratory infection in infants and young children throughout the world (Howard et al. 2000). RSV bronchiolitis is one of the commonest causes of respiratory tract infection leading to respiratory failure (Howard et al. 2000)]. Annually RSV bronchiolitis accounts for 20 000 admissions to hospital in the UK and 90 000 hospitalisations in the USA with associated costs of $300 million per year (Deshpande and Northern 2003; Handforth, Friedland and Sharland 2000). Although primarily a respiratory pathogen of young children,

RSV infects and re-infects adults causing disease in predominatly the elderly, chronic lung disease and immunocompromised patients (Hall 2001). It has been estimated that each year 600,000 deaths occur world-wide that are directly or indirectly attributable to RSV (Howard et al. 2000).

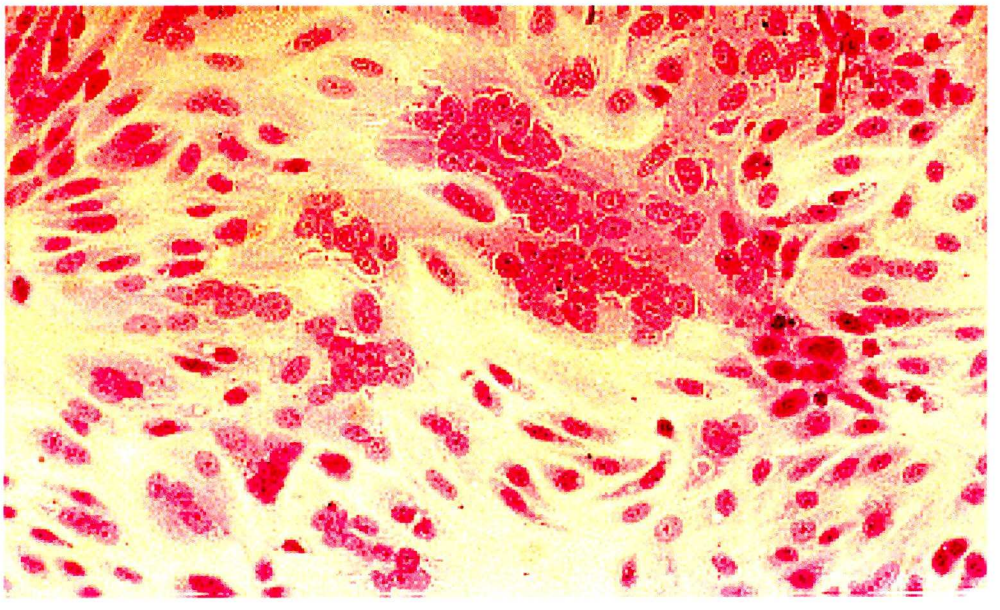

Figure 1.1 A high resolution histology slide of lung tissue showing clumping of RSV-infected host respiratory epithelial cells to form a large multinucleated cell or syncytium, from where respiratory syncytial virus (RSV) acquired its name. (from web.uct.ac.za/depts/mmi/stannard/rsv-cpe2.gif - prepared by Linda M Stannard from photographs taken by Diana Hardie, Dept of Medical Microbiology, University of Cape Town)

1.2 *Classification and Structure*

RSV is a pleomorphic (spherical or filamentous form) enveloped RNA virus 120 - 300nm in size of the order *Mononegavirales*, family *Paramyxoviridae* and genus *Pneumovirus*. RSV contains a non-segmented single-strand negative-sense RNA genome, 15 222 nucleotides in length, that encodes for 9 structural and 2 non-structural proteins.

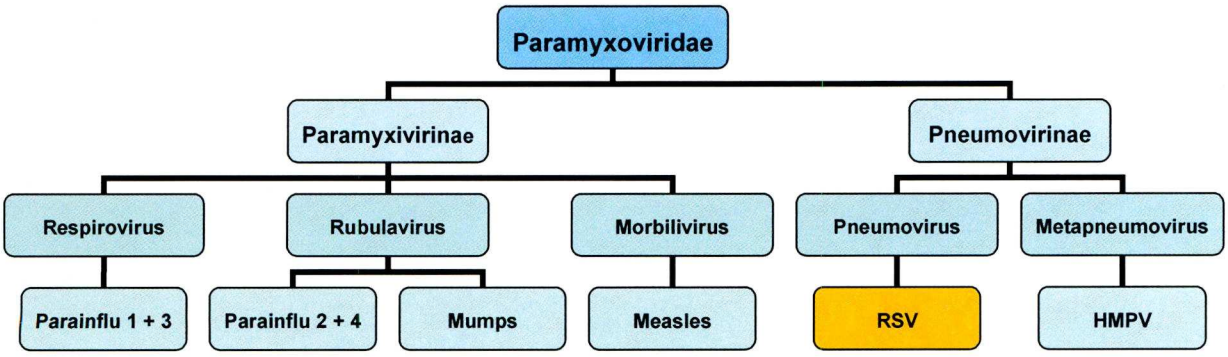

Figure 1.2: Viral taxomony for respiratory syncytial virus (RSV) in the order of family, subfamily, genus and species.

Parainflu = Parainfluenza virus HMPV = Human Metapeumonvirus

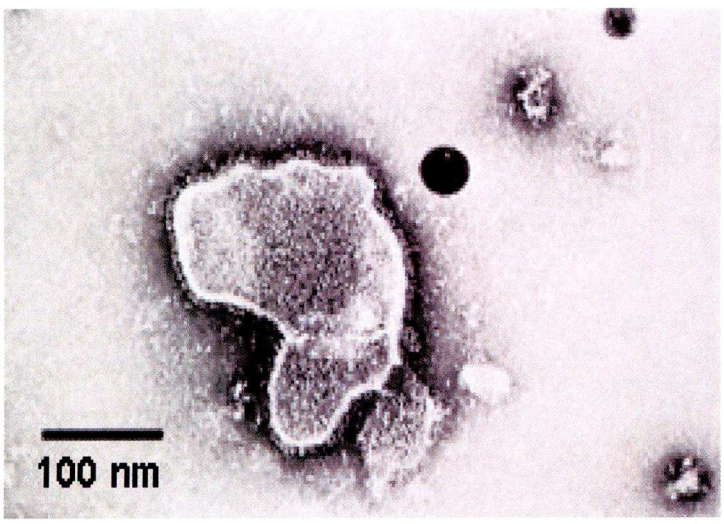

Figure 1.3 An electron micrograph of the respiratory syncytial virus (RSV). The virion is variable in shape and size (average diameter between 120-300nm). (from *the Centers for Disease Control and Prevention, part of the United States Department of Health and Human Services, the image is in the public domain.)*

Two large surface glycoproteins, fusion protein (F) and attachment protein (G), are the major antigenic determinants. Other structural proteins are associated with the viral envelope – matrix proteins, transcription antiterminator protein, small hydrophobic protein (SH), 22 kDa protein; or are found in the nuclear capsule – nucleoprotein (N), large nucleoprotein/polymerase complex (L), phosphoprotein (P), M2-1 and M2-2 matrix proteins. There are 2 non-structural proteins (NS1, NS2) that are unique to pneumoviruses.

There is a lipid bilayer membrane enveloping the matrix protein layer.

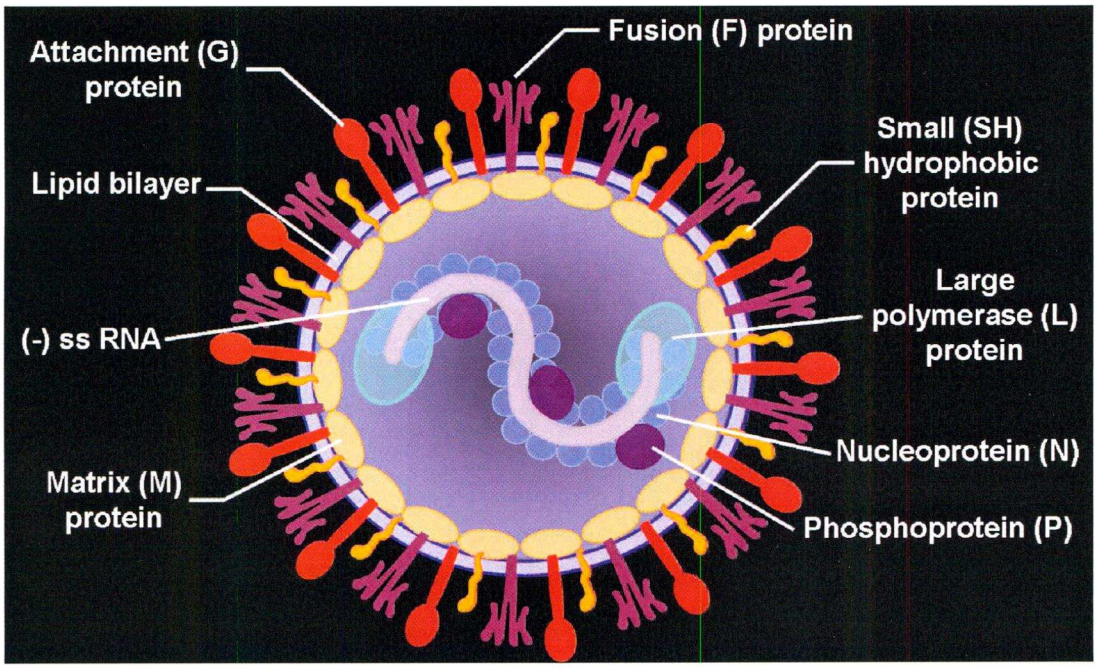

Figure 1.4: Animated diagram of the viral structure of RSV

(from http://www.kuleuven.be/rega/mvr/research.html)

There are two major groups of RSV strains, A and B, that are distinguished by antigenic characteristics, mainly in variations in the G (attachment) protein, as there are few differences in the F (fusion) protein between the strains (Hall 2001; Openshaw 2005; Openshaw and Tregoning 2005; Crowe, Collins and Knipe 2007).

1.3 *Pathogenesis and Pathophysiology*

Viral transmission is by direct inoculation of contagious secretions from hands and self-inoculation of eyes and nose. Transmission requires close or direct contact with large droplets or fomites residing on skin, cloth or clinical surfaces. RSV's incubation period can be 2 - 8 days, usually 2 - 5 days (Hall, Douglas and Geiman 1980; Hall 1980; Hall et al. 1981; Hall and Douglas 1981; Hall 2001; Simoes 1999; Crowe, Collins and Knipe 2007). The virus replicates in nasopharyngeal epithelium and then spreads to lower respiratory tract 1 - 3 days later. RSV infects respiratory epithelial cells by attaching itself to the cell surface by means of a capsular glycoprotein, the G (attachment) protein. A second capsular glycoprotein, the F (fusion) protein, mediates fusion with the epithelial cell membrane along with adjacent cells, resulting in the formation of multinucleated cells – syncytia – for which the virus is named, respiratory syncytial virus (RSV). It is suggested that the small hydrophobic protein (SH) plays a role in both syncytial formation and blocking of cell death / apoptosis. RSV virion assembly occurs at the plasma membrane of infected cells and are released by budding, taking a lipid bilayer membrane derived from the infected host cell with them. Infectious RSV is probably in the filamentous form (Crowe, Collins and Knipe 2007).

RSV has a direct cytopathic effect on respiratory epithelial cells. The characteristic inflammatory process of RSV bronchiolitis leads to loss of cilial motility, submucosal oedema, increased mucus secretion, infiltration by leucocytes, necrosis and sloughing of the respiratory epithelial cells of the small airways, all of which obstructs airflow through the small/distal airways. During expiration this enhances dynamic small/distal airways narrowing producing disproportionate turbulence and decreased airflow causing airtrapping. Further airtrapping can caused by a ball-valve mechanism of airway obstruction due to intraluminal plugging by mucus and cellular debris. Clinically the inflammatory process in the small/distal airways (i.e. bronchiolitis) results in pulmonary hyperinflation, areas of atelectasis, and wheezing due to small/distal airways obstruction.

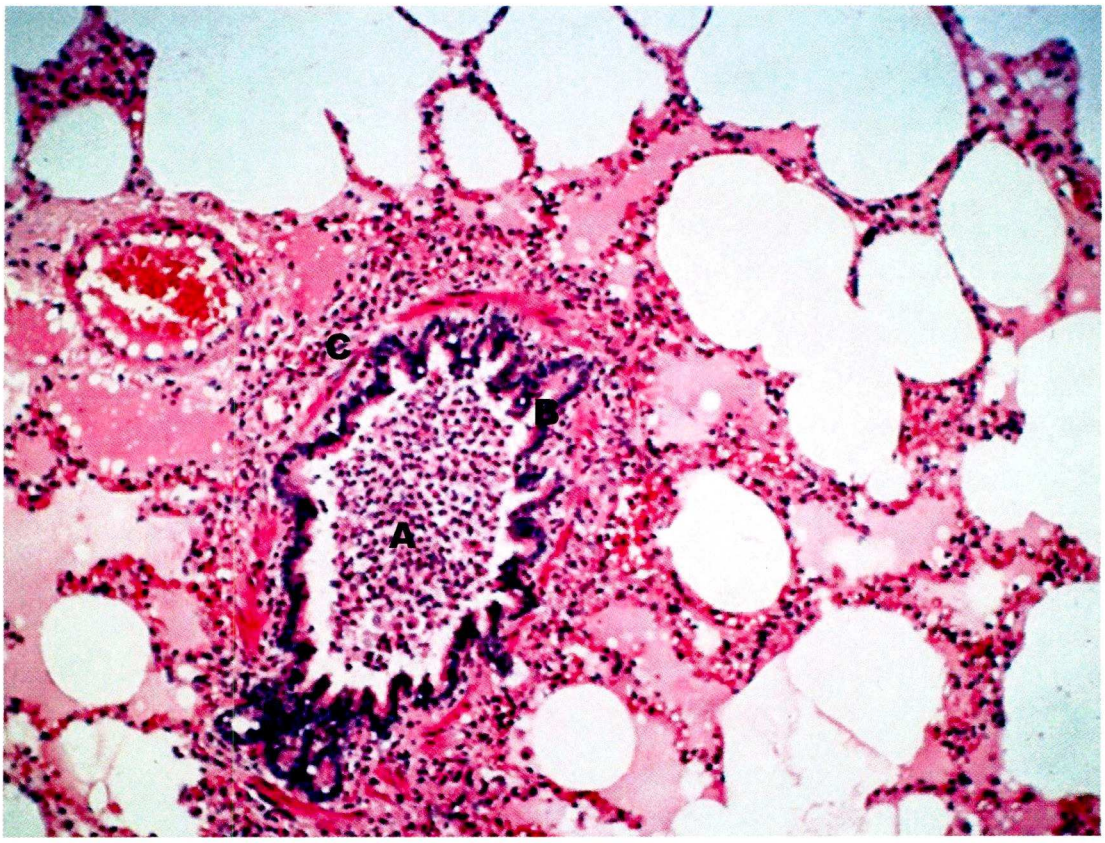

Figure 1.5: Histology slide of human lung tissue demonstrating inflammatory debris (A) obstructing the lumen of a bronchiole (B) with an inflammatory macrophagic infiltrates (C) in the surrounding bronchiolar tissue.

(from www1.imperial.ac.uk/.../respinfect/viralimm/ - Group Leader: Prof Peter Openshaw © Copyright 2009 Imperial College London)

The viral strain (A or B) appears not to be an important factor as studies have failed to show significant differences in virulence and severity of disease between A and B strains (Hall et al. 1990; Kneyber et al. 1996; Hall 2001). The effect of viral load on disease severity is also unclear. Reported data (from the same research team) is conflicting when comparing viral loads in nasals washings between ventilated and non-ventilated children. An initial study (Buckingham, Bush and Devincenzo 2000) demonstrated a higher nasal viral load in the ventilated group, however, a subsequent larger study (Devincenzo, El Saleeby and Bush 2005) failed to find a significant difference. Van Woensel et al found a higher viral load in tracheal aspirates of ventilated infants with "severe RSV LRTI" compared to "mild disease" (differentiaited on

mean airways pressure and oxygenation indices) (van Woensel et al. 2003a). Since viral strain and viral load cannot fully account for variations in disease severity, it is likely that variations in pre-existing structural elements of the distal respiratory tree and the inherent immune response play major roles (Welliver 2003).

The impact of the structural/anatomical factor on the degree of distal airways obstruction is governed by vector properties (for example Pouiseille's Law dictating that the turbulence in the airflow in a cylinder increases to the power of 4 with each decrease in radius), and the physical size/absolute diameter of the distal airway presposing it to intraluminal plugging. Whereas inherent variations in the infected individual's inflammatory response influence the degree of submucosal oedema, increased mucus secretion, and sloughing of epithelial cells. The former factor, anatomical and physical aspects, most probably accounts for the high incidence of respiratory disease in younger smaller individuals with their smaller distal airways (e.g. premature infants) (Simoes 1999; Smyth and Openshaw 2006; Crowe, Collins and Knipe 2007; Simoes 2008). The latter factor, individualistic inflammatory response, continues to fuel much research worldwide.

Clinically airtrapping and atelectasis will increase work of breathing due to increased end expiratory lung volume and decreased lung compliance (Crowe, Collins and Knipe 2007). Respiratory failure is usually the result of worsening lung compliance and fatigue of respiratory muscle. Apnoea, which is common in infants with RSV bronchiolitis, can be secondary to severe lung disease or central in origin. The reported frequency varies from 8% (Vogel et al. 2003) to 21% (Kneyber et al. 1998) of those children admitted to hospital, and up to 20% of those admitted to PICU (Kho et al. 2004).

The respiratory epithelial cells usually recover within 2 – 4 days, but histologically the ciliated epithelial cells take two weeks to regenerate (Hall 2001; Crowe, Collins and Knipe 2007).

1.4 *Immune Response to RSV infection*

Protection against upper (URTI) and lower respiratory tract infections (LRTI) requires a balance between humoral and cellular immunity. Local secretory IgA is the prominent humoral mediator of resistance in the upper respiratory tract, whereas serum IgG provides additional protection in the lower respiratoty tract. The F and G surface glycoproteins are the only RSV proteins to induce protective neutralising antibodies (mainly of the IgG1 subclass) in children (Simoes 1999; Hall 2001; Crowe, Collins and Knipe 2007). In neonates and infants high levels of maternally-derived neutralising antibodies confer some protection (Anderson and Heilman 1995; Hall 2001; Crowe, Collins and Knipe 2007).

Cellular immunity plays the predominant role in combating and recovering from RSV infection, with T-lymphocytic stimulation and response playing an integral function. The antiviral and cell-mediated immune reaction to RSV infection is primarily orchestrated by RSV-infected respiratory epithelial cells and by alveolar macrophages. T helper 1-type cytokines – interferon γ (IFNγ), interleukin type 2 (IL-2), IL-12; T helper 2-type cytokines – IL-4, IL-5, IL-6, IL-10; antiviral interferons – IFNα, IFNβ; and chemokines – C, CC, CXC and CX_3C subgroups, attract and activate leucocytes, especially alveolar macrophages, to the RSV-infected respiratory tract. These cytokines and chemokines may enter the systemic circulation and impact on outlying cells in extrapulmonary sites (Openshaw, Culley and Olszewska 2001; Tripp 2004; Openshaw 2005; Tripp, Oshansky and Alvarez 2005; Culley et al. 2006).

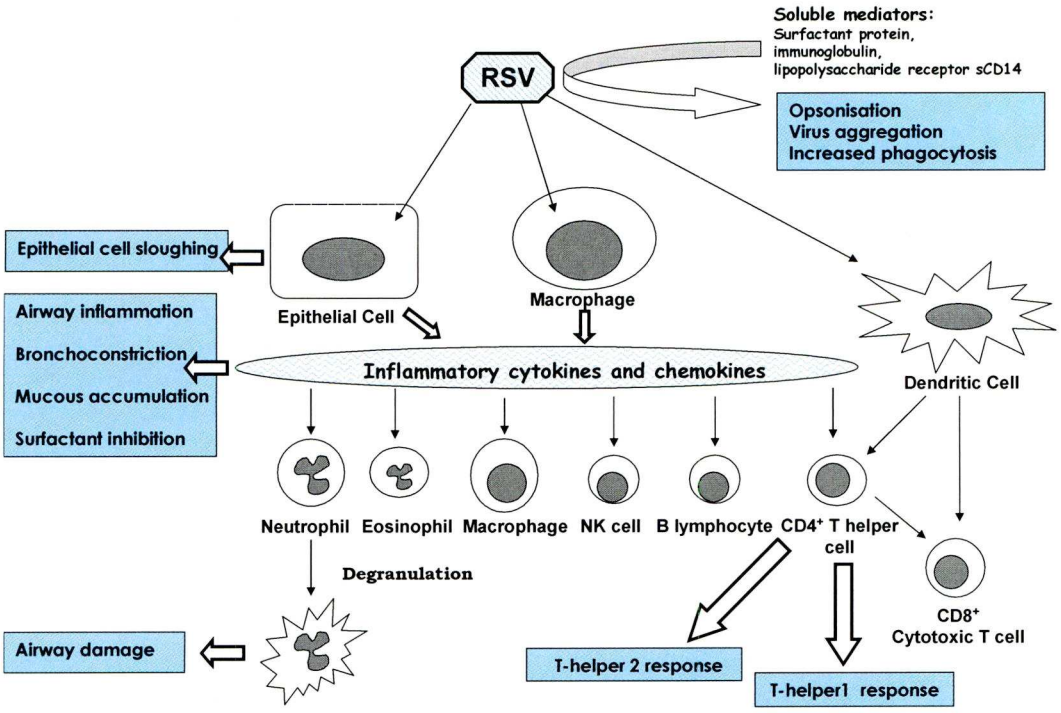

Figure 1.6: Animated diagram of the inflammatory pathways and resultant clinical impact triggered by infection with RSV (adapted, with permission, from a diagram constructed by Dr Steve Brearey, Institute of Child Heath, University of Liverpool)

Much of our understanding of the immune response to RSV infection comes from animal models or studies in children with severe disease (i.e. intubated and mechanically ventilated children from whom respiratory samples can be obtained). Therefore the immunopathogenesis of RSV disease in the great majority of children who develop mild respiratory symptoms, and may not see a doctor or attend hospital, is largely inferred (Tripp 2004; Openshaw 2005; Tripp, Oshansky and Alvarez 2005; Crowe, Collins and Knipe 2007). Severe RSV disease is probably associated with a misdirected immune cascade, characterised by an overexuberant release of inflammatory mediators ("cytokine storm") and infiltration of a range of monocytes and polymorphonuclear cells. Host genetic and acquired factors will manipulate the extent and severity of the immune-augmented response within the respiratory tree, as well as at distant extrapulmonary sites.

In a trial of a formalin-inactivated vaccine in the late 1960's, immunised children suffered more severe disease than controls when they subsequent contracted natural RSV (re)infection (80% required hospitalisation compared to 5% of controls) (Kim et al. 1969). Vaccinated children lacked specific mucosal antibodies; their serum antibodies were deficient in fusion-inhibiting and neutralising activity; some had increased lymphocytic and/or eosinophilic proliferation (Kim et al. 1976; Murphy et al. 1986; Hall 2001; Belshe et al. 2004). These findings suggested that protection against RSV infection necessitates a balance between humoral and cellular immunity. However, the relative contributions of the humoral and cellular immune components in RSV infection and immunity to subsequent RSV infection is still debated (Hall 2001; Openshaw, Culley and Olsewska 2001; Schwarze et al. 2004; Openshaw 2005; Crowe, Collins and Knipe 2007). Effective and appropriately-restrained protection from RSV LRTI most probably requires a viral neutralising response (humoral/antibody + cellular) without an excessive RSV-specific cytotoxic T-lymphocyte response (Simoes 1999; Openshaw, Culley and Olsewska 2001; Schwarze et al. 2004; Openshaw 2005; Crowe, Collins and Knipe 2007).

Primary infection with RSV does not lead to acquired immunity against future infections. Although naturally acquired immunity is neither durable nor complete, it does appear to provide some protection as subsequent infections are less severe (Hall 2001; Openshaw and Tregoning 2005; Crowe, Collins and Knipe 2007). Despite extensive study of the major surface glycoprotein (G), the fusion protein (F), and even the transcription antiterminator matrix protein, the reasons for this lack of acquired immunity remain unclear (Openshaw and Tregoning 2005).

Both laboratory and human studies have demonstrated that immune competent hosts clear the virus following natural RSV infection within 3 weeks, whereas immunocompromised hosts with deficiencies of cellular immunity tend to suffer more severe disease and have prolonged viral shedding (Hall, Douglas and Geiman 1975; Hall 1977; Hall et al. 1986; Wendt and Hertz 1995; Hall 2001; Madhi et al. 2001).

1.5 *Diagnosis*

1.5.1 Clinical diagnosis

The diagnosis of RSV infection for the vast majority of children around the world is made on clinical grounds in diagnosing (RSV) bronchiolitis – coryza, fever, increase respiratory effort, hyperinflation of the chest, wheezing, fine crackles on auscultation, poor feeding and dehydration; which may be backed up by non-specific chest radiographic findings of hyperinflation and patchy atelectasis, and/or below normal oxygen saturations on pulse oximetry (Mulholland, Olinsky and Shann 1990; Mallory et al. 2003; Bordley et al. 2004; Smyth and Openshaw 2006; Bush and Thomson 2007; Yanney and Vyas 2008). Chest radiography (chest xrays) does not differentiate RSV from other viral causes of bronchiolitis or even viral bronchiolitis from bacterial LRTI (Friis et al. 1990; Perlstein et al. 1999; Perlstein et al. 2000; Farah et al. 2002). Additionally, clinical severity and chest radiography changes do not correlate well (Friis et al. 1990; Perlstein et al. 1999; Perlstein et al. 2000; Farah et al. 2002). Although oxygen saturation measurements may influence clinicians in admitting infants with bronchiolitis, the benefits of pulse oximetry in this group have not been proven (Mai et al. 1995; Mallory et al. 2003; Smyth and Openshaw 2006; Bush and Thomson 2007; Yanney and Vyas 2008). Some authors have suggested that certain clinical features like cyanosis and crackles were related to disease severity in bronchiolitis (Mulholland et al. 1990), but others have questioned the validity and reliability of auscultatory findings due to variations in inter-observer agreement (Elphick et al. 2001; Elphick et al. 2004).

1.5.2 Laboratory confirmation of RSV infection

Diagnostic methods for confirming RSV infection in resource-rich countries include viral isolation and culture; direct immunofluorescence and enzyme-linked immunosorbent assays (ELISA) that detect antigen; reverse-transcriptase polymerase chain reaction (RT-PCR) that detect nucleic acid. Seriological testing (acute and convalescent antibody viral titires) is usually clinically unhelpful as seroconversion may take weeks and have a poor

response (Crowe, Collins and Knipe 2007). Rapid antigen detection tests (immunofluorescence and enzyme-linked immunoassays) generally have overall sensitivity and specificity of 80-90% [range 60-95%] (Bordley et al. 2004). RT-PCR is reported to offer greater sensitivity (Fan, Henrickson and Savatski 1998) and multiplex PCR kits that detect several viruses simultaneously are have been developed (Kehl et al. 2001; Kumar et al. 2008). Most commonly nasopharyngeal aspirates are tested, but in intubated patients an endotracheal aspirate or broncho-alveolar lavage can be utilised. The quality of the sample, largely dependent on the sampler's technique and expertise, govern the accuracy of diagnostic testing rather than the site sampled (Bordley et al. 2004; Crowe, Collins and Knipe 2007).

1.5.3 Clinical phenotype

RSV bronchiolitis is the common term for lower respiratory tract disease caused by RSV. Typically the resultant airtrapping will lead to increased end expiratory lung volume and decreased lung compliance compatible with an obstructive lung disease pattern (Simoes 1999, Hall 2001, Bush and Thomson 2007). However, it is appreciated that RSV bronchiolitis/LRTI is a heterogenous disease with some patients having a significant consolidation element and more restrictive lung disease than an obstructive pattern (Isaacs 1998; Frankel and Derish 1999). Some authors have discriminated restrictive from obstructive RSV lung disease on pulmonary function tests (decrease respiratory compliance) (Hammer and Newth 1995, Hammer, Numa and Newth 1997, Newth and Hammer 1998) or ventilatory indices (oxygen index, alveolar-arterial oxygen gradient, mean airways pressure) (Tasker, Gordon and Kiff 2000), in addition to four-quadrant alveolar consolidation on chest radiograph in mechanically ventilated children. In everyday practice (PICU practice included) clinicians cannot strictly dichotomise this heterogeneous lung disease into restrictive and obstructive forms (Isaacs 1998; Frankel and Derish 1999; van Woensel and van Aalderen 2002).

In some European countries and in the USA "RSV bronchiolitis" and "RSV pneumonia" are differentiated clinically by the presence of localised crackles and consolidation on chest radiograph (van Woensel, Kimpen and Brand 2001;

van Woensel and van Aalderen 2002). "RSV pneumonia" is not commonly recognised as a separate clinical entity in the UK (Smyth and Openshaw 2006). Informed clinicians generally appreciate the pneumonic aspects of severe bronchiolitis. In this thesis "RSV bronchiolitis" is not differentiated from "RSV pneumonia", and "RSV bronchiolitis" is synonymous with "RSV LRTI", "RSV lung disease" and "RSV pneumonia".

1.6 *Epidemiology*

RSV affects about 60-70% of infants by the age of 1 year and 90% of children by the age of 2 years, most commonly as bronchiolitis. Peak infection rates occur in infants 6 weeks to 6 months of age. Re-infection occurs frequently (throughout life), but illness tends to be milder (Henderson et al. 1979). Virtually all children have developed antibodies to RSV by 3 years of age (Simoes 1999; Smyth and Openshaw 2006; Crowe, Collins and Knipe 2007).

RSV infection can occur throughout the year, but RSV epidemics occur at predictable annual intervals during the winter months in moderate climates, and during hottest and rainy season in tropical climates (Simoes 1999; Crowe, Collins and Knipe). During their first RSV infection, between 25-40% of infants have bronchiolitic (or pneumonic) signs and symptoms, and 0.5-2.5% require hospitalisation (in developed/resource-rich nations) (Shay et al. 1999; Handforth, Friedland and Sharland 2000; Hall 2009). In the Netherlands RSV bronchiolitis accounts for 3 000 hospitalisations annually, 20 000 in the UK, and 90 000 in the USA (Handforth, Friedland and Sharland 2000; Hall 2001; Deshpande and Northern 2003). Generally the reported hospital admission rates for children under 1 year of age in Europe and the USA is around 30 per 1000 infants (Deshpande and Northern 2003; Dayan et al 2004; Levine et al. 2004; Smyth and Openshaw 2006). Incidence and hospitalisation rates for RSV bronchiolitis in resource-limited areas are lacking owing to the paucity of epidemiological studies and cost-saving restrictions in laboratory confirmation of RSV infection (Hussey et al. 2000; Madhi et al 2006; Morrow et al. 2006; Smyth and Openshaw 2006; Argent 2008).

Approximately 3-10% of hospitalised RSV-infected infants (and about 1% of all RSV bronchiolitics) develop respiratory failure and require admission to PICU (Navas et al. 1992; Brooks et al. 1999; Buckingham et al. 2001; Smyth and Openshaw 2006). Amongst the children from resource-rich countries admitted to PICU with RSV infection, the reported incidence of those requiring mechanical or non-invasive ventilation varies from 50-100% depending more on the country, than the region or unit (Navas et al. 1992; Buckingham et al. 2001; Leclerc et al. 2001; Duttweiler, Nadal and Frey 2004; Randolph, Reder and Englund 2004; Kneyber et al. 2005; Kneyber, Plötz and Vincent 2007; Thorburn et al 2006).

The mortality rate for those hospitalised with RSV infection is usually low at 1 – 3%, but the mortality rate increases in children with severe bronchiolitis requiring intensive care management (Buckingham et al. 2001; Shay et al. 2001; Holman et al. 2003; Thompson et al. 2003; Thorburn 2009). It has been estimated that annually in the USA up to 2700 deaths are caused by RSV (Thompson et al. 2003; Handforth, Friedland and Sharland 2000). RSV is the most common viral cause of death in children below 5 years of age and especially in infants less than 1 year old (Thompson et al. 2003). World-wide each year 600,000 deaths occur that are directly or indirectly attributable to RSV (Thompson et al. 2003; Fleming, Pannell and Cross 2005)].

Both A and B strains circulate concurrently within a RSV season. There are distinct genotypes within the strains which vary in dominance within a community from year to year (Hall et al. 1990; Peret at al. 1998; Peret et al. 2000). This may account for the frequency of re-infection as any immunity to previous genotypes is evaded (Hall et al. 1990; Peret at al. 1998; Peret et al. 2000). Convincing differences in virulence and severity of clinical disease between A and B strains have not been demonstrated (Hall et al. 1990; Kneyber et al. 1996; Hall 2001).

1.7 *Severity of Disease and Risk factors*

The vast majority of children with RSV bronchiolitis will be treated in the community, with only up to 3% requiring admission to hospital (Shay et al. 1999; Handforth, Friedland and Sharland 2000; Deshpande and Northern 2003; Bush and Thomson 2007). Most studies therefore define severe disease by the requirement for hospitalisation (Navas et al. 1992; Langley et al. 1997b; Handforth, Friedland and Sharland 2000; Howard et al. 2000; Deshpande and Northern 2003; Holman et al. 2003; Purcell and Fergie 2004; Bush and Thomson 2007; Jansen et al. 2007). Although a number of clinical scoring systems have been proposed (and frequently utilised in bronchiolitis studies), none have proved better than clinical judgement (Fitzgerald and Kilham 2004; Plint et al. 2004; Bush and Thomson 2007). Even national guidelines on indications for hospital referral, for example those produced by the Scottish Intercollegiate Guidelines Network or the American Academy of Pediatrics, still rely on clinical judgement in interpreting clinical features and recognition of risk factors that predispose to severe disease (Baumer 2007, Carraro, Zanconato and Baraldi 2009).

Risk factors that are associated with increased severity of disease can be divided into host and environmental risk factors. Host factors include chronological age less than 6 weeks, prematurity, chronic lung disease, congenital heart disease, neurological disease, and immunodeficiency (Navas et al. 1992; Arnold et al. 1999; Shay et al. 1999; Simoes 1999; Handforth, Friedland and Sharland 2000; Hall 2001; Law, Carbonell-Estrany and Simoes 2002; Nielsen et al. 2003; Purcell and Fergie 2004a; Smyth and Openshaw 2006; Wilkesmann et al. 2007). Additionally there are some indicators (for example surfactant protein D gene polymorphism) to suggest host genetic predisposition to severe RSV infection (Lahti et al. 2002; Goetghebuer et al. 2004; Smyth and Openshaw 2006). Dual respiratory viral infections (RSV in addition to other respiratory viruses) have also been shown to increase disease severity as indicated by the need for PICU admission and mechanical ventilation (Semple et al. 2005; Richard et al. 2008). Environmental factors include poverty, overcrowding, malnutrition, and exposure to postnatal tobacco

smoke, older siblings, nursery attendance (Gurkan et al. 2000; Law et al. 2002; Smyth and Openshaw 2006; Yanney and Vyas 2008).

RSV infection of the respiratory tree can lead to respiratory failure due to deteriorating lung compliance and increasing fatigue of respiratory muscles, resulting in the need for respiratory support. Apnoea, secondary to severe lung disease or central in origin, occurs in 8% to 21% of infants admitted to hospital with RSV bronchiolitis (Kneyber et al. 1998; Vogel et al. 2003). Frequently it is an indication for PICU admission and is reported in up to 20% of those admitted to PICU (Kho et al. 2004).

Within the large group of children hospitalised with RSV infection there is a smaller subgroup (3 – 10%) which require intensive care treatment. It is recognised that this group has more severe RSV disease (Navas et al. 1992; Brooks et al. 1999; Frankel and Derish 1999; Simoes 1999; Smyth and Openshaw 2006). Many clinicians and researchers, and certainly the critical care community, regard this group as truly reflecting severe RSV disease (Tasker et al. 2000; Buckingham et al 2001; Leclerc et al. 2001; Bont and Kimpen 2002; Kneyber et al. 2002; Davison et al. 2004; Duttweiler et al. 2004; Randolph et al. 2004; Kneyber et al. 2005, Kneyber et al. 2007; Argent 2008; Richard et al. 2008; Tasker 2008).

► For this thesis, PICU admission has been used as the marker of severe RSV disease.

1.8 *Therapeutic Options in PICU*

"Oxygen is vitally important in bronchiolitis and there is little evidence that any other treatment is useful" – Reynolds and Cook (Reynolds and Cook 1963).

It is 45 years since this statement by Reynolds and Cook and the clinical situation essentially remains the same. Maintaining adequate oxygenation and hydration is the mainstay of largely supportive treatment in RSV bronchiolitis (Cooper, Banasiak and Allen 2003; Bordley et al. 2004; King et al. 2004; Scarfone 2005; Checchia 2008).

1.8.1 Oxygen

There are no randomised controlled trials or systematic reviews investigating the use of oxygen in LRTI, let alone RSV bronchiolitis. Evidence for the use of oxygen supplementation is extrapolated from case-control studies that show hypoxaemia as a risk factor for near-fatal asthma (Turner et al. 1998). It is generally recommended that oxygen saturation levels are maintained above 92% (Fitzgerald and Kilham 2004; Baumer 2007; Bush and Thomson 2007; Carraro et al. 2009; Rojas, Granados and Charry-Anzola 2009).

1.8.2 Bronchodilators

Bronchodilators, generally $ß_2$ agonists, are commonly prescribed in children with bronchiolitis in Europe and North America (Plint et al. 2004; Scarfone 2005). Heterogenicity in study design and the bronchodilator used complicate comparisons between studies (King et al. 2004). Systematic reviews demonstrated modest improvement in clinical scores following bronchodilator therapy in mild to moderate bronchiolitis, but no improvement in oxygenation and hospital admission rate (Flores and Horwitz 1997; King et al. 2004; Gadomski and Bhasale 2006). There are few studies investigating the benefit of bronchodilators in children with RSV bronchiolitis requiring mechanical ventilation (Mallory et al. 1989; Hammer and Newth 1995; Derish et al. 1998; Levin et al. 2008). Any transient improvement in measured lung functions did not translate to significant and sustained clinical benefit or decrease in length of ventilation (Argent 2008). Their routine use in ventilated patients, as with non-ventilated patients, remains unsupported (Argent 2008; American Academy of Pediatrics 2006).

1.8.3 Adrenaline (Epinephrine)

Nebulised adrenaline with its ß adrenergic bronchodilator effect, along with the α adrenergic effect of pulmonary vasoconstriction and reduction in oedema, has been considered useful and used in the treatment of bronchiolitis. Although studies on nebulised adrenaline in bronchiolitis show it to have a good safety profile, short-term improvement in clinical scores when compared

to both placebo and salbutamol failed to translate into clinically significant improvement in oxygenation or hospital admission rates (Lowell et al. 1987; Sanchez et al. 1993; Menon, Sutcliffe and Klassen 1995; Reijonen et al. 1995; Lodrup Carlsen and Carlsen 2000; Bertrand et al 2001; Numa, Williams and Dakin 2001; Patel et al. 2002; Ray and Singh 2002; Hariprakash et al. 2003; Hartling et al. 2003; Wainwright et al. 2003; Mull et al. 2004; Langley et al 2005; Plint et al. 2009). A Cochrane review of this treatment confirmed these findings (Hartling et al. 2004). Routine use is generally not recommended (Fitzgerald and Kilham 2004; Scarfone 2005).

1.8.4 Corticosteroids

The rationale for the use of corticosteroids (inhaled, oral or intravenous) comes from their acknowledged benefit in other obstructive airways disease, such as asthma, and their ability to inhibit the immune response which contributes to the pathogenesis of RSV disease (Openshaw, Dean and Culley 2003; van Woensel et al. 2003a; Openshaw and Tregoning 2005; Hall 2007). Heterogenicity in study design and the corticosteroid administered make comparisons between studies difficult. A systematic review of 13 trials (1198 patients) failed to demonstrate benefit in outcome (usually requirement for hospital admission, requirement for respiratory support, or length of stay in hospital) from systemic corticosteroids (Patel et al. 2004). A systematic review of 5 studies (374 patients) failed to show benefit in outcome (hospital (re)admission) from inhaled corticosteroids (Blom et al. 2007). A recent randomised controlled trial examined whether dexamethasone treatment to previously healthy infants presenting with moderate to severe bronchiolitis for the first time reduced the need for hospitalisation (Corneli et al. 2007). No significant difference was found between the corticosteroid and placebo groups. Likewise a randomised placebo-controlled trial in 2009 examining whether treatment with dexamethasone for children ventilated with severe RSV disease impacted on cytokine concentrations in tracheal aspirates and disease severity (days of ventilation, PICU and hospitalisation) failed to demonstrate a difference between the dexamethasone and placebo groups (Somers et al. 2009).

Three studies investigated the the role of corticosteroids in ventilated children with RSV bronchiolitis (van Woensel et al. 1997; Buckingham et al 2002; van Woensel et al. 2003b). Different corticosteroid and treatment durations were used. No significant difference in duration of mechanical ventilation between the treatment and placebo arms were found in any of the 3 trials. A meta-analysis combining these 3 trials (66 patients) showed no overall effect on duration of mechanical ventilation or on duration of hospitalisation (Davison et al. 2004).

Currently van Woensel et al. are performing a third multicentre randomised controlled trial (the Steroid Treatment in Artificially ventilated children with RSV infection [STAR] trial) investigating the potential benefit of corticosteroids in ventilated children with RSV bronchiolitis (personal communication). Its results are awaited, but I am sceptical as to whether they will impact on future PICU management of severe RSV bronchiolitis.

1.8.5 Methylxanthines

Data from uncontrolled trials suggest that there may be some benefit in using methylxanthines, such as theophylline and caffeine, in infants with bronchiolitis-associated apnoeas (Ramesh and Samuels 2005). A randomised, double blind, placebo controlled trial to determine whether treatment with aminophylline will reduce the need for ventilatory support in infants with bronchiolitis is currently in progress in Melbourne, Australia (Royal Children's Hospital Melbourne 2007).

1.8.6 Chest physiotherapy, nebulised hypertonic saline

Three trials have failed to demonstrate compelling evidence of the benefit of chest physiotherapy in bronchiolitis, as borne out by a systematic review (Perrotta, Ortiz and Roque 2007).

A recent meta-analysis of 4 trials investigating the effect of nebulisation with hypertonic 3% saline solutions vs. 0.9% saline solutions suggested that nebulised 3% saline solutions may hold some benefit - reduced length of

hospital stay and a decreased clinical severity score. However, none of the studies included mechanically ventilated children (Zhang et al. 2008).

1.8.7 Ribavirin

Ribavirin is a purine nucleoside analogue that is believed to interfere with viral nucleic acid function. Ribavirin has activity against RSV, influenza, and hepatitis C (Hall 1983; Hall et al. 1986; Crowe, Collins and Knipe 2007). Ribavirin is expensive, difficult to deliver as the nebulised droplets adhere to the ventilatory circuit, and teratogenic (therefore potentially toxic to both the patient and the treating team) (Fitzgerald and Kilham 2004). Systematic reviews have failed to show any convincing effect in the acute setting (Ventre and Randolph 2007).

Three trials have studied ribavirin in ventilated patients (Smith et al. 1991; Meert et al 1994; Guerguerian et al. 1999). Meta-Analysis in a Cochrane review of the 104 patients that were mechanically ventilated in these 3 trials showed that aerosolised ribavirin was associated with a significant decrease in the duration of mechanical ventilation of 1.2 days (95%CI -0.2 to -3.4, p = 0.03) (Randolph and Wang 2000). However, saline was utilised as the placebo in two studies (Meert et al. 1994; Guerguerian et al. 1999) and sterile water in the third (Smith et al. 1991). Sterile water has the serious potential side-effect of induction of bronchospasm which potentially confounds interpretation of data from this study. When the 28 patients from this study were excluded from the meta-analysis, the difference in duration of mechanical ventilation became insignificant (1.08 days, 95%CI 0.67 to – 2.83, p = 0.2) (Davison et al. 2004). Additionally, in the three trials the between-study variation in length of mechanical ventilation of the control groups ranged from 5.3 days (Guerguerian et al. 1999) to 9.9 days (Smith et al. 1991) confounds interpretation.

Because of high cost, safety concerns, challenges in delivery, and weakness with trial data, ribavirin is usually only considered in immunocompromised children in the PICU setting in Europe (Kneyber, Moll and de Groot 2000).

1.8.8 Surfactant

Endogenous surfactant lowers the surface tension within the alveoli at the alveolar-capillary membrane level. The rationale for exogenous surfactant comes from the findings of low levels of surfactant phopholipids and proteins, along with reduced surfactant function, in children with severe bronchiolitis (Tibby et al. 2000; Leclerc 2001; Ventre, Haroon and Davison 2006; Kneyber and Plötz 2007). Due to its endotracheal route of administration exogenous surfactant can only be considered in intubated children. Three randomised controlled trials, (79 patients in total) investigating its efficacy have been published. Two used porcine surfactant and no placebo [Luchetti et al. 1998; Luchetti et al. 2002), and the other bovine surfactant and an air placebo (Tibby et al. 2000). A meta-analysis of the 3 studies showed a nonsignificant reduction in duration of mechanical ventilation by 2.6 days (95%CI -5.34 to 0.18 days; p = 0.07) and significant reduction in PICU days bi 3.3 days (95%CI -6.38 to -0.23 days; p = 0.04). However, between-study variation in length of mechanical ventilation of the control groups in the three trials of 5.8 days (Luchetti et al. 2002), 7.1 days (Tibby et al. 2000) and 8.9 days (Luchetti et al. 1998), along with variation in study design, confounds effective interpretation. A systematic review highlighted the inadequacy of available data (Ventre, Haroon and Davison 2006). Any future large randomised controlled trial will be hampered by the need for multiple centres to obtain adequate numbers and the expense of exogenous surfactant.

1.8.9 Heliox (helium / oxygen mixture)

The pathophysiological rationale for heliox is that with a density one-seventh that of air it would result in decreased resistance to airflow (Gupta and Cheifetz 2005). A prospective, randomised, double-blind study in 20 infants with bronchiolitis found a significant improvement in a modified clinical asthma score after 1 hour of heliox use (Cambonie et al. 2006). A single randomised controlled trial in 18 PICU patients (though only 13 were randomise d) reported improvement in a clinical asthma score during heliox delivery, but did not report length of PICU stay (Hollman et al. 1998). Only 7 children received ventilatory support – mechanical ventilation 1, continuous positive airways

pressure (CPAP) 6, so it is difficult to glean any relevant PICU interpretation from this data. One trial investigating the effect of various concentrations of heliox on mechanical ventilation in 10 infants with RSV bronchiolitis demonstrated no beneficial effect (Gross, Spear and Peterson 2000). A multicentre, randomised, double-blind placebo-controlled study in 39 infants with bronchiolitis found no difference in the need for intubation between controls and the study group following 24 hours of heliox use (Liet et al. 2005). A recent Dutch study of 9 infants with RSV bronchiolitis reported reduced "respiratory system resistance", but failed to address benefits in clinical terms (i.e. need for intubation, duration of ventilation, length of stay) (Kneyber et al. 2009).

1.8.10 Inhaled Nitric Oxide (iNO)

Inhaled nitric oxide (iNO) by nature of its route of administration produces vasodilation in the bronchial tree, thereby enhancing the blood flow and the ventilation-perfusion quotient. There is a single study examining the effect of inhaled nitric oxide on respiratory mechanics in 12 ventilated infants with RSV bronchiolitis (Patel et al. 1999). It concluded that iNO had no apparent bronchodilator effect in the majority of acutely ill infants with RSV bronchiolitis and did not appear to provide any additional benefit over the use of salbutamol.

A Cochrane review of randomised controlled trials (535 ventilated children and adults) analysed the effect of iNO in acute hypoxemic respiratory failure (Sokol, Jacobs and Bohn 2004). It found that iNO did not demonstrate any statistically significant effect on mortality or ventilator-free days, and only transiently improved oxygenation in patients with hypoxemic respiratory failure.

1.8.11 Recombinant human DNAse (rhDNAse)

Intraluminal mucus plugs in the distal airways is an important pathophysiologic feature in RSV bronchiolitis. DNA released by degenerating leucocytes is present in these mucus plugs and contributes to their increased viscocity and

adhersiveness (Nasr et al. 2001). By cleaving this released DNA, rhDNAse can help liquify the mucus. Anecdotal data suggested that rhDNAse was effective in infants with severe RSV bronchiolitis (Merkus et al. 2001; Nasr et al. 2001). A multicentre, randomised, double-blind placebo-controlled study in 224 infants with RSV bronchiolitis found that administration of rhDNAse did not reduce the length of hospital stay, duration of supplemental oxygen, and number requiring intensive care or mechanical ventilation (Boogard et al. 2007).

1.8.12 Interferon, Erythropoietin, Vitamin A

There are 2 studies assessing the efficacy of vitamin A (Neuzil et al. 1994; Quinlan and Hayani 1996), and single studies each of interferon (Chipps, Sullivan and Portnoy 1993) and erythropoietin (Jacobs, Lyons and Brilli 2003) in RSV infection. None of the studies showed a significant difference between the study groups and controls.

1.8.13 Respiratory support

If despite oxygen supplementation children develop respiratory failure artificial respiratory support (non-invasive or mechanical ventilation) may become necessary. The application of continuous positive airway pressure (CPAP) keeps the airways open and thereby facilitates expiratory flow, improves compliance, reduces work of breathing and enhances gas exchange. There is supportive evidence that delivery of CPAP via a mask or nasal prongs may reverse impending respiratory failure and avoid intubation (Beasley and Jones 1981; Soong, Hwang and Tang 1993; Larrar et al. 2006; Thia et al. 2007; Cambonie et al. 2008; Shah, Ohlsson and Shah 2008). Intubation and mechanical ventilation (positive pressure ventilatory support) is the mainstay of supportive therapy for children with RSV-induced respiratory failure due to worsening lung compliance, imminent respiratory collapse secondary to exhaustion, or apnoea and respiratory arrest (Yanney and Vyas 2008). Already in the 1980s, retrospective studies confirmed the effectiveness of mechanical ventilation in bronchiolitis-associated respiratory failure (Outwater

and Crone 1984; Lebel et al. 1989). Unfortunately there are no randomised controlled trials on the level of positive end-expiratory pressure (PEEP) or ventilatory strategies (for example, volume-controlled vs pressure controlled, or high frequency vs conventional ventilation) for ventilated children with RSV-induced respiratory failure (Leclerc et al. 2001; Kneyber and Plötz 2007; Greenough 2009). Perhaps this is because RSV bronchiolitis is a heterogeneous lung disease with varying obstructive and restrictive elements, rather than a homogemous clinical entity (Isaacs 1998; Frankel and Derish 1999; van Woensel and van Aalderen 2002; Kneyber and Plötz 2007). When maximum conventional mechanical ventilation or high frequency oscillatory ventilation (HFOV) fail to stabilise or reverse deteriorating oxygenation (and ventilation), extracorporeal life support (ECLS) / extracorporeal membrane oxygenation (ECMO) is the last port of call for these refractory cases. Survival rates of ECLS/ECMO for RSV bronchiolitis are better than other indications for ECLS/ECMO and range from 71% (Flamant et al. 2005) to as high as 96% (Khan et al. 1995), with a low rate of neurological sequelae (Steinhorn and Green 1990).

1.9 *Preventive Treatments*

1.9.1 RSV Immunoglobulin

Hyperimmune RSV immunoglobulin (RSVIG) and monoclonal RSV immunoglobulin augment neutralising antibodies and are used for immunoprophylaxis in high risk patients (Hall 2001; Meissner and Long 2003; American Academy of Pediatrics 2003; Meissner et al. 2004; Smyth and Openshaw 2006; Bush and Thomson 2007). They have been shown to reduce hospital admissions from RSV bronchiolitis (Impact-RSV Study Group 1998; Wang and Tang 2000; Fuller and Del 2006). Both are expensive, offer partial protection, and require monthly intravenous (RSVIG) or intramuscular (monoclonal RSV immunoglobulin) injections (Fuller and Del 2006). Their prohibitive expense has lead to many cost-effectiveness analyses and the restriction of use to targeted high risk groups (Kamal-Bahl, Doshi and

Campbell 2002; American Academy of Pediatrics 2003; Rackham, Thorburn and Kerr 2005; Fuller and Del 2006).

The use of hyperimmune RSV (polyclonal) immunoglobulin has fallen out of favour due to its intravenous route, the intravenous volume required, an increased risk of adverse outcomes in infants with cyanotic heart disease, and possible inactivation of live vaccines (for example measles-mumps-rubella) (American Academy of Pediatrics 2003). RSVIG is not licensed for treatment in the UK.

Palivizumab, the first humanised monoclonal antibody against the surface F glycoprotein in RSV, is the immunoprophylactic agent generally favoured and has been studied extensively (Wang and Tang 2000; Fuller and Del 2006). Palivizumab has been shown to reduce RSV-related hospital admission by 55% in preterm infants born at less than 32 weeks gestation (1002 palivizumab recipients vs 500 controls) (Impact-RSV Study Group 1998) and by 45% in infants born with significant congenital heart disease (639 palivizumab recipients vs 648 controls) (Feltes et al. 2003). Despite this reduction in hospital admission for serious RSV disease, the cost-benefit balance for infants born at more than 32 weeks gestation or with congenital heart disease is still debated intensely (Joffe et al. 1999; Clark et al. 2000; Kamal-Bahl, Doshi and Campbell 2002; American Academy of Pediatrics 2003; Prais, Schonfeld and Amir 2003; Wegner et al. 2004; Yount and Mahle 2004; Bala, Ryan and Murphy 2005; Embleton, Harkensee and McKean 2005; Heikkinen et al. 2005; Prais et al. 2005; Meberg and Bruu 2006; Sunnegardh 2006; Geskey, Thomas and Brummel 2007; Nuijten, Wittenberg and Lebmeier 2007). Although the RSV-IMpact trial examining the efficacy of palivizumab in preterm infants demonstrated an overall reduction in hospitalisation of 55% compared to controls, it did not impact on the number requiring PICU admission (1.3% vs 3%) or the number requiring mechanical ventilation (0.2% vs 0.7%) (Impact-RSV Study Group 1998). Similarly, despite an overall reduction in hospitalisation of 45% compared to controls in the trial examining the efficacy of palivizumab in children with congenital heart disease, the number requiring PICU admission (2% vs 3.7%) or the number requiring mechanical ventilation (1.3% vs 2.2%) was not signiciantly different (Feltes et al. 2003). Post

palivizumab licensure studies comparing the number of children requiring PICU admission and mechanical ventilation in the RSV seasons prior to palivizumab to those in the RSV seasons following its prophylactic use have found no significant reductions following the introduction of palivizumab (Pedraz et al. 2003; Prais et al. 2005).

Three trials have examined the use of RSVIG (Rodriguez et al. 1997) or palivizumab (Malley et al. 1998) in infants admitted to PICU with RSV infection – 125 children; 103 mechanically ventilated. There was no difference in ventilator days, length of PICU stay, length of hospital stay, adverse reactions or mortality compared to controls in any of the studies.

The Joint Committee on Vaccination and Immunization (a UK Department of Health advisory body) made the following recommendations regarding indications for palivizumab prophylaxis (June 2005):

1. Children under 2 years of age with chronic lung disease (oxygen dependency for at least 28 days from birth), on home oxygen or who have had prolonged use of oxygen.
2. Infants less than 6 months of age who have left to right shunt haemodynamically significant congenital heart disease and/or pulmonary hypertension.
3. Children under 2 years of age with severe congenital immuno-deficiency.

The indications for the use of palivizumab at the Royal Liverpool Children's Hospital - Alder Hey are displayed in Table 2.2. They reflect widely accepted high risk subgroups and other guidelines internationally (Meissner and Long 2003; Yount and Mahle 2004; Embleton, Harkensee and McKean 2005).

1.9.2 Vaccination

The first RSV vaccine produced in the 1960's was a formalin-inactivated vaccine. Even though it produced high serum antibody levels it resulted in

worse bronchiolitis following RSV infection in the vaccinated group than the control non-vacinated children (Simoes 1999; Handforth, Friedland and Sharland 2000; Bush and Thomson 2007). Development of an effective RSV vaccine is being actively explored and is a high research priority (Hall 2001; Smyth and Openshaw 2006: Bush and Thomson 2007). A RSV vaccine needs to offer better protection than that from natural infection and be effective in the first weeks of life when maternally-acquired anti-RSV antibodies are still present. Live attenuated vaccines have the potential advantages of being delivered intranasally and inducing both a local mucosal and a systemic immune response. However, they tend to be unstable, too virulent and revert back to wild-type virus (Handforth, Friedland and Sharland 2000; Hall 2001; Smyth and Openshaw 2006). Vaccines produced from purified viruses, recombinant vectors, and plasmids containing complementary DNA of the viral genome that generally target the F (fusion) and G (attachment) transmembrane glycoproteins are being trialled (Simoes 1999; Handforth, Friedland and Sharland 2000; Hall 2001; Kneyber and Kimpen 2004; Smyth and Openshaw 2006: Bush and Thomson 2007).

1.10 *Current Research*

1.10.1 Overall aims of Current Research

The initial trigger for the current research was an anecdotal observation that a number of the children ventilated with RSV bronchiolitis appeared to have raised liver enzymes / hepatic transaminases. This led to a 2 year retrospective study of 55 children (52 mechanically ventilated) admitted to PICU with severe RSV bronchiolitis in which it was found that hepatitis, as reflected by raised alanine transaminase (ALT) and asparate transaminase (AST) levels, was a common extrapulmonary manifestation associated with severe RSV disease (Eisenhut and Thorburn 2002). The hypotheses for many of the subsequent studies evolved from the findings of the prior studies.

The overall aim of this thesis is to investigate the clinical impact of severe RSV disease on children, with the need for admission to PICU being the marker of severe RSV disease.

1.10.2 Specific aims of separate parts of the study

1.10.1.1 To determine the relationship between hepatic transaminase levels and disease severity, and the aetiology of the elevated transaminase levels in children with severe RSV disease.

1.10.1.2 To determine the prevalence of myocardial involvement and its relationship with disease severity in children with severe RSV bronchiolitis.

1.10.1.3 To investigate whether severe RSV bronchiolitis is associated with reduced right ventricular function in infants without congenital heart disease, and to determine whether reduced right ventricular function is associated with myocardial damage, elevated transaminase levels, and disease severity.

1.10.1.4 To determine the incidence of pulmonary bacterial co-infection and its impact on mortality and morbidity in children with severe RSV bronchiolitis.

1.10.1.5 To determine the mortality rate and the risk factors for death in children admitted to PICU with RSV infection.

1.10.1.6 To determine the incidence of nosocomial (acquired) RSV infection in critically ill children, and the impact of nosocomial RSV infection on mortality and morbidity.

2 Patients and Methods

2.1 *Patients*

2.1.1 Setting

Children admitted to the PICU at the Royal Liverpool Children's Hospital - Alder Hey, a tertiary university-affiliated, multi-disciplinary, regional referral centre, were studied. The PICU is a 20-bed facility with an annual admission rate of approximately 1100 children. Cardiac and medical patients each account for 40% of the PICU admissions and 20% are surgical (all subspecialities represented). The overall mortality rate is 4.5%, with a predicted mortality of 6.25% using Paediatric Index of Mortality (PIM) (Shann et al 1997) and a standardized mortality rate of 0.72.

2.1.2 Inclusion criteria

All children with RSV infection, confirmed on RSV antigen testing, immunofluorescence and/or culture that required PICU admission between June 1999 and May 2009 (i.e. encompassing ten consecutive RSV seasons – October to March) were studied.

2.1.3 Study design

The data were collected prospectively from June 2001 onwards (the most recent eight RSV / winter seasons studied) and retrospectively from June 1999 – June 2001 (the first two RSV seasons studied).

PICU admission was utilised as the marker of severity of disease.

2.1.4 Study population

The PICU at the Royal Liverpool Children's Hospital is a multidisciplinary tertiary and regional referral centre. The referral/catchment area (North West

England + North Wales) has a paediatric population (0 - 16 years age) of 1 474 900 (Office for National Statistics (England & Wales) Census 2001).

Annually usually between 220 and 380 children are admitted to the Royal Liverpool Children's Hospital with confirmed RSV bronchiolitis (Figure 2.1).

Annually approximately 50 RSV-infected children are admitted to the PICU at Royal Liverpool Children's Hospital (Figure 2.2 and Table 2.1).

Children admitted to PICU may have been admitted via the Accident & Emergency (A&E) Department in the Royal Liverpool Children's Hospital, from one of our children's wards, or may originally have presented to another hospital (usually a district general / secondary level hospital) prior to transfer to the regional PICU.

Children were referred to PICU at the clinical discretion of the primary medical team responsible for the care of the child. Children for whom intensive care was requested were usually in respiratory failure or impending respiratory collapse and required respiratory support (most often mechanical ventilation).

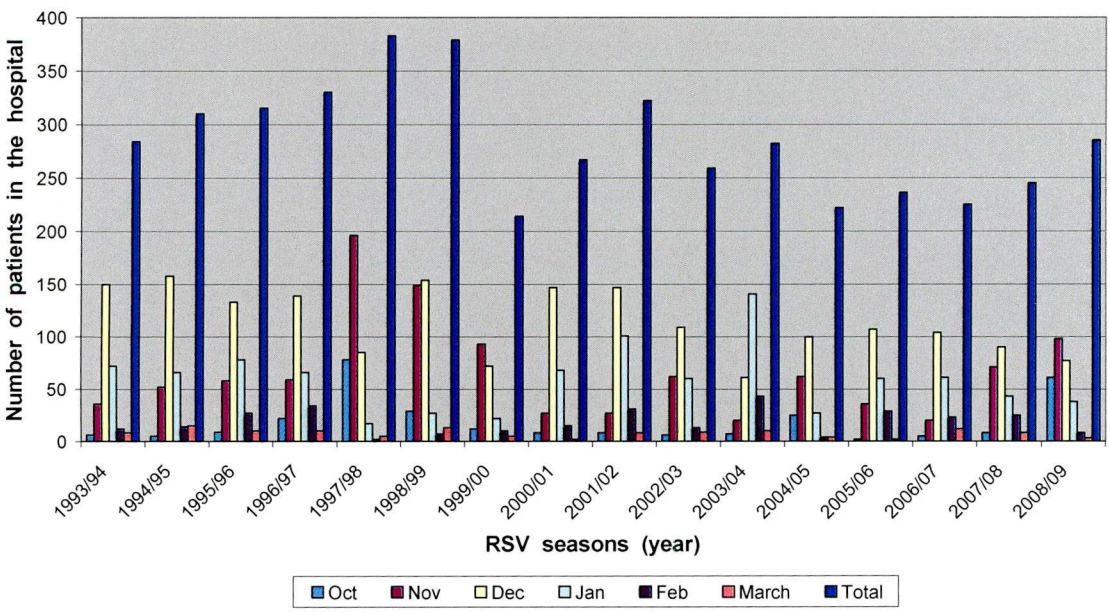

Figure 2.1: The number of RSV bronchiolitis children admitted each month and in total to the Royal Liverpool Children's Hospital: 1993 - 2009.

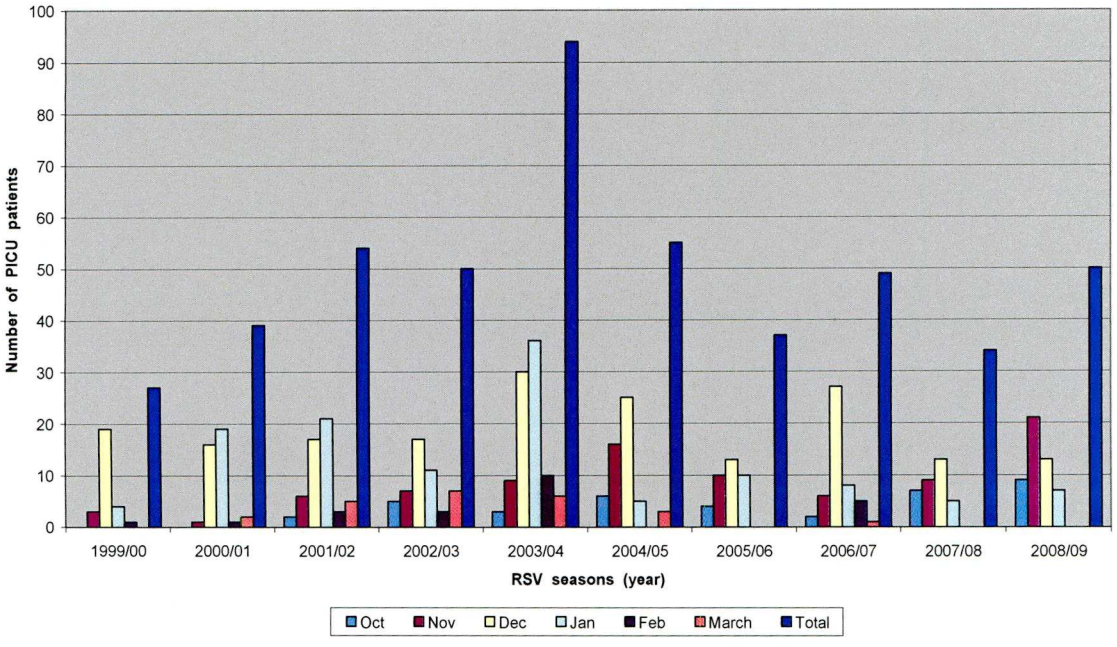

Figure 2.2: The number of RSV-infected patients admitted each month and in total to PICU during the ten RSV seasons: 1999 - 2009.

Table 2.1: RSV-infected patients admitted each season to PICU during the ten RSV seasons: 1999 – 2009. median (interquartile range)

RSV seasons	Number	Age	Length of Stay	Deaths
1999 - 2000	28	1.6 (1.1 : 5.8)	8 (6 : 9)	1
2000 - 2001	39	3.8 (1.7 : 5.8)	6 (3 : 7)	7
2001 - 2002	54	1.5 (0.9 : 7.4)	7 (5 : 13.5)	8
2002 - 2003	50	2.0 (0.9 : 5.4)	6 (4 : 8)	3
2003 - 2004	94	3.6 (1.2 : 16.1)	6 (4.5 : 9)	9
2004 - 2005	55	4.6 (1.8 : 17.8)	6 (4 : 12.5)	4
2005 - 2006	37	2.4 (1.3 : 6.4)	5 (4 : 9)	3
2006 - 2007	49	2.4 (1.3 : 6.5)	4 (3 : 7)	0
2007 - 2008	34	2.6 (1.4 : 8.0)	4 (3 : 7)	5
2008 - 2009	50	3.1 (1.5 : 14.4)	5 (3 : 8)	2

2.1.5 Research & Ethics

All studies had been approved by the Royal Liverpool Children's Hospital NHS Trust Research and Development Directorate – as required, following full processing, approval was granted by the Liverpool Children's Research Ethics Committee, or the Liverpool Children's Research Ethics Committee waived the need for formal ethical approval for those studies that in their opinion fell into the realms of audit. The latter were registered with the Clinical Audit Department, a division of the Directorate of Research and Development at the Royal Liverpool Children's Hospital NHS Trust.

2.2 *Clinical Management*

2.2.1 Medical management

Following PICU admission the child was reviewed promptly, by one of the six PICU consultants who are responsible for the management of critically ill children in the Royal Liverpool Children's Hospital. Subsequent management depended on the condition and clinical progress of the child. The PICU consultants work closely as a team, patients are discussed regularly and similar management strategies are used.

2.2.2 Therapeutics

Adequate hydration and oxygenation is the mainstay of medical management in (RSV) bronchiolitis. No specific RSV treatments (for example bronchodilators, corticosteroids, surfactant, ribavirin, etc.) were employed in our PICU in the RSV infected patients. All the study patients received standard PICU management which included early enteral feeding.

2.2.3 Respiratory support

It is national policy that all children who require intensive care and mechanical respiratory support are transferred to the regional PICU. Intubation was performed by our PICU retrieval team at the referring hospital, in our A&E

department, or in one of the hospital wards prior to PICU admission. Alternatively, the anaesthetic team of the referring hospital intubated some of the patients preceding the arrival of our PICU retrieval team.

All forms of mechanical ventilation (conventional and high frequency) and continuous positive airways pressure (CPAP) support are carried out on the PICU at the Royal Liverpool Children's Hospital. Patients were given sufficient oxygen to maintain oxygen saturation at a minimum of 93% when measured by continuous pulse oximetry. Ventilation strategies were tailored to the individual's evolving respiratory disease and not protocolised indescriminantly. Permissive hypercapnia was tolerated in order to minimise barotrauma and volume trauma to the lungs. Muscle relaxants were not routinely used.

Non-invasive CPAP support was infrequently employed on our PICU because it is usually performed on our 14-bed high dependency unit (HDU) which is separate from the PICU. More recently some of the regional secondary-level hospitals have been performing non-invasive CPAP support within their own paediatric HDU/wards.

Children requiring extracorporeal life support (ECLS) / extracorporeal membrane oxygenation (ECMO) because of deteriorating oxygenation despite maximal ventilatory support were transferred to one of four national centres.

The timing of extubation was judged clinically and not influenced at all by any of the studies.

2.2.4 Antimicrobial policy

The empiric use of antibiotics in children with severe bronchiolitis was at the discretion of the attending consultant. Patients with signs of infection received intravenous cefotaxime (50 mg/kg/dose 4 times daily) as first line therapy for 48 hours whilst awaiting culture results. Clinical status on presentation governed whether supplementary intravenous cover with an aminoglycoside, gentamicin (7 mg/kg/day - single daily dose) was added. Antibiotics were rationalised or stopped once culture and sensitivity results became available.

Ribavirin therapy is only considered in immunocompromised children.

RSV immunoprophylaxis with the humanised monoclonal antibody, palivizumab is used at the Royal Liverpool Children's Hospital - Alder Hey on a very selective basis. The indications for the use of palivizumab are displayed in Table 2.2. They reflect widely accepted high risk subgroups and other guidelines internationally (Meissner and Long 2003; Meissner et al. 2004; Yount and Mahle 2004; Embleton, Harkensee and McKean 2005).

Table 2.2: Indications for palivizumab treatment / immunoprophylaxis at the Royal Liverpool Children's Hospital - Alder Hey, Liverpool, UK .

The following high risk patient groups can be considered for palivizumab treatment if the consultant considers them to be at particular risk should they get RSV infection.

1. Long term ventilated (LTV) patients (UK LTV working party 1998)
    - All children on LTV aged less than 12 months at the start of the RSV season
    - Children on LTV aged less than 24 months with additional co-pathology:
        a. Heart disease/pulmonary hypertension
        b. Intrinsic lung disease (*as reflected by oxygen dependency*)

2. Congenital heart disease in children under 6 months with either
    a. A haemodynamically significant left-to-right shunt
    b. Pulmonary hypertension

3. Children under 24 months with either
    - Severe congenital immunodefiency
    - Chronic Lung Disease (CLD) using oxygen at home

4. Children in the first 6-12 months of life born at under 35 weeks gestation

    Only if there are other factors considered by the consultant specialist to significantly increase the risk of hospitalisation

2.3 *Diagnosis*

2.3.1 Microbiologic sampling

Diagnostic samples of nasopharyngeal aspirates (for RSV detection) and lower airway secretions (for bacterial culture and viral detection) were taken through the endotracheal tube using sterile precautions (de Blic et al. 2000) on admission to PICU and thereafter when clinically indicated.

From January 2002 onwards RSV surveillance samples (nasopharyngeal or endotracheal aspirates) were taken on all children during the RSV season (October – March) on admission and twice a week while on PICU.

Nasopharyngeal and endotracheal aspirate samples were collected by specialist respiratory physiotherapists or PICU staff members.

Broncho-alveolar lavage (BAL) sampling was performed by specialist PICU respiratory physiotherapists. Prior to routine bronchial toilet a sterile suction catheter was passed down the endotracheal tube. Two 1ml/kg aliquots of sterile 0.9% saline were instilled through the suction catheter, immediately followed by aspiration with constant pressure into a mucus trap. BAL was performed immediately after endotracheal intubation in children intubated in our hospital and on arrival in the PICU - generally within three hours of endotracheal intubation for those retrieved from other hospitals.

Surveillance samples of throat and rectum were obtained on admission and then twice weekly, in keeping with the routine surveillance practice in our unit.

2.3.2 Laboratory procedures

2.3.2.1 Viral

Respiratory tract secretions were tested by the Directigen™ RSV test [Becton Dickinson microbiology systems, Maryland, USA] (1999 – 2004) or NOW™ RSV test [Binax Inc., Maine, USA] (2005 – 2009). These are *in vitro* immunochromatographic assays for the rapid and qualitative detection of RSV fusion protein antigen directly from nasopharyngeal and tracheal specimens.

The specimen is applied directly to the RSV test kits (Binax) and following 15 minutes incubation, the test window is assessed for reactivity. RSV antigen present in the sample reacts to bind anti-RSV conjugate antibody. The resulting antigen-conjugate complexes are captured by immobilised anti-RSV antibody in the sample line of the test strip. Immobilsed anti-RSV antibody in the control line captures a visualising conjugate, allowing reading of a positive result as both a pink-to-purple sample line and control line. Commercially available rapid antigen tests are designed to detect the most commonly occurring RSV strains, but may not detect all RSV strains.

Samples negative for RSV using the rapid antigen tests were cultured using Rmix shell vial culture techniques at the Public Health Laboratory (Aintree, Liverpool, UK).

Immunofluorescence for RSV was performed using SimulFluor™ reagents (Chemicon/Millipore, Massachusetts, USA) at the Public Health Laboratory (Standards Unit HPA UK 2007).

2.3.2.2 Bacterial/Yeast

Diagnostic or clinical samples were processed immediately in a qualitative and semi-quantitative way using standard microbiological methods. Broncho-alveolar lavages were centrifuged at 1200xg for 10 minutes. All but 0.5ml of the supernatant was removed and the centrifuged deposit re-suspended in the remaining fluid. A sterile calibrated loop was used to plate out 0.01ml of the BAL sample. Thereafter the agar plates were incubated. Less than 10 colonies on the agar plate equated to $<10^3$ CFU/ml; between 10-100 colonies equated to 10^3-10^4 CFU/ml; >100 colonies to $>10^4$ CFU/ml; and >1000 colonies to $> 10^5$ CFU/ml of BAL. Chocolate, MacConkey, Staph 110, and blood agars were used for bacterial isolation; and Sabuoraud agar for fungi. Standard methods for identification, typing, and sensitivity patterns were used for all micro-organisms (Brook 1979, Baselski and Wunderlink 1994, Standards Unit HPA UK 2009).

2.3.2.3 Biochemistry

Arterial blood gases were measured when clinically relevant from indwelling arterial lines (as is standard in PICU management) on blood gas analysers within the PICU – Rapidlab 1265, Bayer Diagnostics Limited, Sudbury, UK.

Urea and creatinine levels, transaminase levels (alanine and aspartate aminotransferase levels), and C-reactive protein (CRP) levels were determined daily by photometric analysis using the Abbott Architect c8000 routine biochemistry analyser in the Department of Clinical Biochemistry at the Royal Liverpool Children's Hospital. Normal ranges for urea was 2.3 - 6.4 mmol/l and for creatinine 24 - 52 umol/l. Normal ranges for alanine aminotransferase was 9 - 36 IU/l and for aspartate aminotransferase 15 - 58 IU/l. Elevated C-reactive protein level was defined as a level of > 10mg/l (Hicks and Soldin 1995).

Cardiac troponin T (cTnT) levels, as a marker of myocardial damage (Immer et al 1997), were determined on the day of echocardiographic assessment with a one-step monoclonal sandwich immunoassay (Roche Diagnostics Ltd) in the Department of Clinical Biochemistry at the Royal Liverpool Children's Hospital. The cTnT immunoassay has a lower limit of detection of 10 pg/ml, with minimal cross reactivity with cardiac troponin I of 0.002%, and skeletal troponin T of 0.001% (Roche Diagnostics GmbH: TroponinT STAT data sheet Mannheim, Germany 1999). Elevated levels were defined as values of >10 pg/ml.

2.3.2.4 Cardiac assessments

Pulse wave Doppler echocardiographic assessment (using Vivid 5 or Vivid 7, General Electric Healthcare, Milwaukee, USA) was performed by Paediatric Cardiologists on admission in enrolled patients - measurements included: pulmonary valve ejection time, tricuspid valve closure time, left ventricular end diastolic and systolic dimension, left ventricular fractional shortening, and Tei index. Measurements were repeated three times per patient and average values calculated. The two cardiologists reviewed and analysed the recordings independently. The average of their calculated values was utilized.

Electrocardiography (ECG) monitoring was routinely undertaken throughout the admission with any abnormalities leading to formal tracing and analysis.

2.4 *Definitions and Formulae*

RSV infection – any child admitted to the PICU that tested positive for RSV on RSV antigen testing, immunofluorescence and/or culture from respiratory secretions.

RSV disease – any child that tested positive for RSV on RSV antigen testing, immunofluorescence and/or culture from respiratory secretions that had either clinical bronchiolitis or apnoeas related to RSV infection.

Community-acquired RSV infection - Children with RSV bronchiolitis confirmed on RSV antigen testing, immunofluorescence or viral culture from respiratory tract secretions on admission to hospital.

Hospital ward-acquired RSV infection - Children who were RSV negative (or from whom no samples were taken as they had no signs of bronchiolitis) on admission to hospital and who then became RSV positive five or more days after admission to hospital (Hall et al. 1978; Raymond and Aujard 2000; Thorburn et al. 2004).

PICU-acquired RSV infection - Children who were RSV negative (or from whom no samples were taken as they had no signs of bronchiolitis) on admission to PICU and who then became RSV positive five or more days after admission to PICU (Hall et al. 1978; Raymond and Aujard 2000; Thorburn et al. 2004).

RSV-attributable deaths – deaths were considered directly attributable to RSV if the patients were still RSV positive when they died or when the RSV infection contributed to the adverse clinical course leading to death (Simon et al. 2008, von Renesse et al. 2009).

PIM-predicted mortality – the paediatric index of mortality (PIM) predicted mortality was calculated according to the original description (Shann et al. 1997). The clinical and physiological features are scored at the time of first contact with the intensive care staff (if children are retrieved to the unit, at the referring hospital).

Standardised mortality ratio (SMR) – the ratio of actual deaths compared to the number predicted (in this thesis derived from PIM-prediced mortality).

Oxygen Index (OI) – mean airways pressure (MAP) x FiO_2/PaO_2

Ventilation Index (VI) – respiratory rate x $PaCO_2$ x peak inspiratory pressure / 1000.

CFU/ml – colony forming units of a single bacterial species per ml of diagnostic sample.

2.5 *Analytic Methods*

Results were expressed as a percentage of the total study population; median and inter-quartile ranges (IQR) were used to describe the demographic distributions. Prediction of mortality using paediatric index of mortality (PIM) was obtained on the patient's first contact with the PICU team (Shann et al. 1997). Standardised mortality ratio (SMR) is the ratio of actual deaths compared to the number predicted (in this study derived from PIM score). Continuous data were analysed using the Wilcoxon-Mann-Whitney (W-M-W) test. Categorical data were analysed using Fisher's exact or McNemar's test. Multivariate analysis was performed using linear and logistic regression analysis. Statistical calculations were performed with the Statistical Program for Social Science (SPSS) version 11.0, 13.0 or 15.0 (Chicago, Illinois) and Epi-Info version 6.04 (CDC, Atlanta). A p value < 0.05 was considered statistically significant. All p-values were two tailed. Relative Risk (RR) with 95% confidence intervals (95% CI) calculations was performed with Confidence Interval Analysis software (CIA 2.1.2) from *Statistics with Confidence*, editors: DG Altman, D Machin, TN Bryant, MJ Gardner; BMJ Books, London (2000).

3 Hepatic transaminase levels and disease severity in children with severe RSV disease

3.1 *Introduction*

Previously in our PICU we had noted anecdotally that a number of the children ventilated with RSV bronchiolitis appeared to have raised liver enzymes / hepatic transaminases. This led to an earlier 2 year retrospective study of 55 children (52 mechanically ventilated) admitted to PICU with severe RSV bronchiolitis in which it was found that hepatitis, as reflected by raised alanine transaminase (ALT) and asparate transaminase (AST) levels, was a common extrapulmonary manifestation associated with severe RSV disease (Eisenhut and Thorburn 2002). Transaminase levels were elevated in 49% (27 of 55) of these children. The duration of ventilation was significantly longer in the children with elevated transaminase levels compared to those with normal transaminase levels ($p = 0.02$, 2-sample t-test for unequal variance). This association was independent of potentially confounding factors such as medication administered, hypoxic event before admission, chronic lung disease and congenital heart disease.

Those children with elevated transaminases appeared to have more severe disease as reflected by their longer duration of ventilation. A possible explanation was that they were co-infected with a second pathogen (most likely viral) that resulted in more severe respiratory disease and caused hepatitis. An alternative explanation was the possibility that RSV itself held the potential to cause hepatitis. There are few reports in the medical literature of hepatic involvement in RSV disease. There is a report of RSV being isolated from the liver tissue of a 7-month old infant with bronchiolitis and extrahepatic biliary atresia (Nadal et al. 1990). The only other report was that of hepatitis in 3 children with severe bronchiolitis (Tristam et al. 1988). However, in all these described cases there was dual infection with RSV and cytomegalovirus

(CMV) or adenovirus.

It was proposed that coincidental infection with other viruses as described previously (Tristam et al. 1988) may have caused the hepatitis or myocarditis which led to an increase in transaminase levels and more severe disease. A prospective cohort study in patients admitted to the PICU with RSV bronchiolitis was therefore conducted to investigate hepatic transaminase levels and their relationship with disease severity, and whether there was evidence on concomitant viral hepatitis.

3.2 *Patients and Methods*

3.2.1 Setting:

20-bed regional multidisciplinary tertiary paediatric intensive care unit (PICU) in university-affiliated children's hospital.

3.2.2 Objectives:

1) To investigate the origin of transaminase level elevation by looking at ALT/ AST ratios in patients with and without congenital heart disease, and cardiac Troponin T (cTnT) levels in patients with a ratio of ALT to AST < 1 indicative of a possible cardiac origin of the transaminase elevation. Cardiac troponin T has been shown to be a more sensitive marker of myocyte injury in myocarditis than conventional cardiac enzyme levels (Lauer et al. 1997).
2) To compare disease severity as judged by duration of ventilation, inotrope use and mortality in infants ventilated for RSV positive lower respiratory tract infection with and without elevated transaminase levels.
3) To investigate for concomitant infection with common hepatitis viruses and respiratory viruses, which have been associated with hepatitis.

3.2.3 Patients:

In a prospective observational study consecutive children ventilated with severe RSV bronchiolitis, confirmed on RSV antigen testing, immunofluorescence and/or culture, during the annual RSV winter epidemic between October 2001 and March 2002 were enrolled. The local Research and Ethics Committee of Royal Liverpool Children's Hospital NHS Trust (Institutional Research Ethics Review Board) had waived the need for informed consent by parents or guardians of children included in the study.

3.2.4 Measurements:

3.2.4.1 Virology:

RSV antigen was detected following standard methods with an antigen ELISA in nasopharyngeal and endotracheal secretions. This was followed by cell culture of the secretions using standard virological techniques at the Public Health Laboratory (Aintree, Liverpool, UK) to improve sensitivity of detection of RSV. For additional detail see 2.3.2.1. Further virology studies in children with elevated transaminase levels were conducted at the Virology laboratory of the Public Health Laboratory Services at University Hospital Aintree and at the PCR laboratories at Manchester Children's Hospital NHS Trust. Common paediatric hepatitic viruses were sought, as well other respiratory viruses we had found to cause a severe bronchiolitic picture with extra-pulmonary clinical impact. Hepatitis A virus IgG and IgM levels, HbsAg, HbcAntibodies, Hepatitis C virus and Epstein Barr virus antibodies were measured by standard ELISA-methods. Detection of adenovirus, influenza A and B-, parainfluenza-I, II, and III viruses was by tissue culture systems using HEP-2- and rhesus monkey kidney- as well as MRC-5- (embryonic human lung) cells. Cytomegalovirus DNA was determined by PCR with the Taqman™ System by Roche™.

3.2.4.2 Biochemistry

All study participants had, as part of the daily routine biochemical profile, ALT and AST levels determined. ALT levels were considered to be elevated if

above 36 IU/l and AST levels if they were above 58 IU/l (Hicks and Soldin 1995). Cardiac troponin was utilised to differentiate between hepatic or cardiac origin of elevated transaminases. Cardiac troponin T has been shown to be a more sensitive marker of myocyte injury in myocarditis than conventional cardiac enzyme levels (Lauer et al. 1997). Cardiac troponin (cTnT) levels were measured when AST levels were higher than ALT levels. A ratio of ALT/AST <1 was considered to be indicative of possible myocardial involvement and a ratio of ≥1 was considered to be indicative of hepatitis as described previously (Kanda et al 1995). Transaminase levels were measured by photometric analysis and cTnT levels were measured with a standard chemoluminescence assay (Elecys^{TM}) at the laboratories for Clinical Chemistry at the Royal Liverpool Children's Hospital - Alder Hey. Cardiac TnT levels were considered to be elevated if >10pg/ml.

3.2.4.3 Analytic Methods:

Data on age, gestational age at birth, history of Neonatal Intensive Care Unit admission, history of chronic lung disease or congenital heart disease, duration of symptoms before admission, duration of ventilation, inotrope use, death attributable to respiratory syncytial virus infection, Paediatric index of mortality (PIM) scores, transaminase and cTnT levels and results of viral studies arranged were collected with a standardized data collection form. The Paediatric Index of mortality and resulting predicted probability of death were calculated from variables extracted on admission (Shann et al. 1997). They comprised of specified diagnosis on admission (yes/no), elective admission (yes/no), mechanical ventilation (yes/no), systolic blood pressure (mmHg), base excess in arterial or capillary blood (mmol/l), ratio FiO_2/PaO_2 ($mmHg^{-1}$) and pupils fixed to light (yes/no).

Statistical data analysis was performed with Excel for Word for Windows (Office 2000^{TM}) and Epi info version 6.04b (produced by The Division of Surveillance and Epidemiology, Epidemiology Program Office, Centre for Disease Control, Atlanta, USA). For comparison of categorical data chi-square tests with Yates correction and Fisher exact tests were used. For continuous data the two sample Student t-test was used after logarithmic

transformation of data if skewness of data was detected. Differences with a p-value of < 0.05 were considered to be statistically significant. All p-values were two-tailed. Multivariate analysis was performed using logistic regression analysis.

3.3 *Results*

During the study period 54 children with RSV bronchiolitis were admitted to PICU and 48 of them were enrolled in our study. Reasons for the 6 excluded from analysis were: PICU discharge before investigations could be organised, n = 3; PICU discharge before RSV detected on tissue culture (RSV antigen/ELISA negative patients), n = 2; ventilation due to upper airways abnormality/obstruction and not due to RSV infection, n = 1. Elevated transaminase levels (ALT and/or AST) were found in 22 (46%). Demographic and clinical characteristics of patients with and without elevated transaminase levels are shown in Table 3.1.

Thirteen out of 15 (86%) patients with congenital heart disease (CHD) and 9 out of 33 (27%) patients without CHD had elevated transaminase levels ($p<0.01$). The types of cardiac lesions in the 15 children in the study with CHD were: ventricular septal defects (7); transposition of the great arteries (2); tetralogy of Fallots (2); double outlet right ventricle (2); hypoplastic left ventricle (2); truncus arteriosus (1); coarctation of the aorta (1); persistent ductus arteriosus (1); anomalous pulmonary venous drainage (1). Eleven of the 15 children with CHD had had complete or partial correction of their underlying cardiac lesion before entry into the study. Six of the 15 children with CHD were cyanotic at the time of PICU admission, and 4 were in cardiac failure clinically.

The ALT/AST ratio was ≥ 1 in 6 (27%) patients. ALT/AST ratio was <1 in 16 patients (73%). Of patients with ALT/AST ratio < 1, 62% were patients with congenital heart disease. The proportion of patients with CHD amongst patients with predominantly elevated AST levels was not statistically significant different from the proportion of patients without CHD ($p = 0.65$). Time course of ALT levels in patients with elevated levels is illustrated in Figure 3.1. Two

outliers had ALT levels which peaked at 3676 and 1490 IU/l on days 5 and 6 after admission respectively. Both patients died, however elevated ALT did not necessarily correlate with poor outcome. In Figure 3.2, AST levels of patients with and without CHD were compared. AST levels in patients with CHD were significantly higher than in patients without CHD on day 2 and 3 after admission.

Cardiac troponin T levels were elevated in 6 (50%) out of the 12 of patients with an ALT/AST ratio <1. In 4 of such patients a cTnT level was not performed. The elevation of cTnT levels at its peak was to a median of 610pg/ml (range 160 to 2720pg/ml). All patients with elevated cTnT levels had underlying CHD. Three out of the 6 patients who died and 5 out of the 11 patients on inotropes had elevated cTnT levels. All patients that died, and all but one patient with inotrope requirements, had underlying CHD.

Virological studies revealed a simultaneous infection with a second virus in only one patient – Influenza A infection was detected in nasopharyngeal secretions, with no evidence of recent infection with any of the other viruses investigated.

Multiple logistic regression analysis utilizing transaminase levels as the dependent variable and entering CHD, inotrope use, and death as covariates with backward stepwise (likelihood ratio) regression, revealed that CHD emerged as the only factor with a significant ($p < 0.01$) change in -2log likelihood on removal from the model at the last step.

Table 3.1: Demographic and clinical characteristics of patients ventilated with RSV bronchiolitis with and without elevated transaminase levels.

	Patients with elevated transaminase levels (n = 22)	Patients with normal transaminase levels (n = 26)	p-value
Age on admission (months)	2.0 (1.0;6.5)[a]	1.5 (0.7;7.0)	0.61[b]
Gestational age at birth (months)	36.3 (5.1)[c]	38.4 (2.4)	0.15[d]
Duration of symptoms before admission (days)	2.7(2.4)[c]	3.3 (2.4)	0.38[e]
History of Neonatal Intensive Care	7	6	0.72[f]
Chronic lung disease of prematurity	3	1	0.32[g]
Congenital heart disease (CHD)	13	2	<0.01[g]
Predicted probability of death in %, according to PIM model	9.3 (8.1;10.5)[h]	8.3 (7.1;9.4)	0.71[b]
Duration of ventilation (days) in all patients	10.6 (9.4;11.7)[a]	3.5 (2.8;4.2)	<0.01[b]
Duration of ventilation (days) in patients without CHD	7.9 (6.7; 9.1)[a]	3.5 (2.8;4.2)	<0.01[b]
Patients requiring inotropes (n = 11)	9	2	<0.01[g]
Number of deaths in each group	6 (27%)	0 (0%)	<0.01[g]

[a] median (IQR)
[b] two sample t-test for unequal variance after logarithmic transformation of data
[c] arithmetic mean (SD)
[d] two sample t-test for unequal variance
[e] two sample t-test
[f] chi-square test with Yates correction
[g] Fishers exact test with two tailed p-values
[h] geometric mean (95% CI).

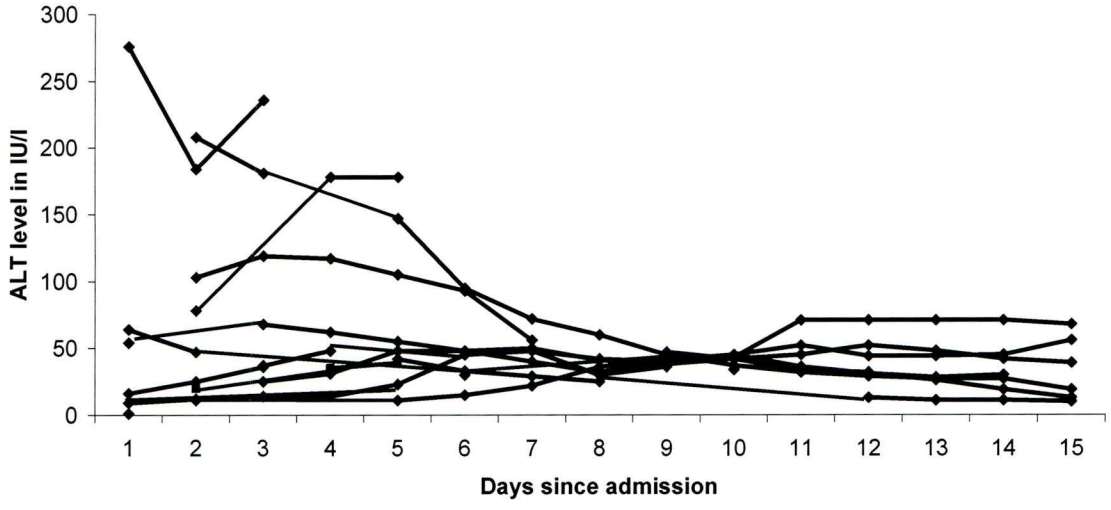

Figure 3.1: Alanine aminotransferase (ALT) levels in all patients ventilated with RSV bronchiolitis with elevated ALT-levels (n = 16).

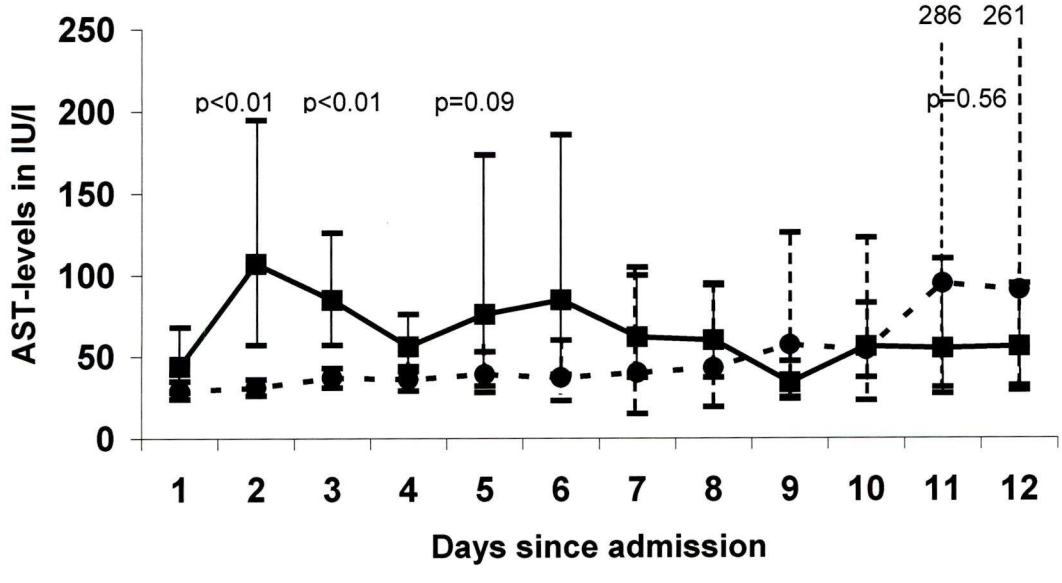

Figure 3.2: AST levels in children with —— (n = 15) and without - - - (n = 33) congenital heart disease ventilated with RSV bronchiolitis (geometric means and 95% CI; p-values are from two tailed, two sample t-test for unequal variance after log_{10}-transformation of data).

3.4 *Discussion*

Hepatic transaminase levels were elevated in 46% (22 out of 48) of children ventilated with severe RSV bronchiolitis. There are several possible explanations for this finding. Virus-induced hepatitis, ischaemic hepatitis or hypoxic hepatitis were possible causes in patients with elevated hepatic transaminase and normal cTnT levels. None of the medication used on the PICU was hepatotoxic and a previous study (Eisenhut and Thorburn 2002) demonstrated that there was no association between medications used and hepatitis in children ventilated for severe RSV bronchiolitis. The lack of infection with either known hepatitis viruses or respiratory viruses known to cause hepatitis may suggest that RSV itself caused the hepatitis. Conclusive proof of hepatic RSV infection would require a liver biopsy and this is inappropriate on ethical grounds.

RSV has been isolated from both liver tissue and the myocardium previously (Nadal et al. 1990; Fishaut, Tubergen and McIntosh 1980). A case report of hepatopulmonary syndrome in viral hepatitis caused by hepatitis A virus suggested that a link between hypoxia (due to intrapulmonary shunting) and hepatitis, even without fulminant hepatic failure (Regev et al. 2001). A previous retrospective study in our PICU (Eisenhut and Thorburn 2002) indicated that there was no evidence of hypoxic events before admission being associated with elevated transaminase levels. In another study, hypoxic hepatitis has only been seen in severe hypoxia (arterial $PO_2 \leq 6.kPa$) (Henrion et al. 1999). Hypoxic hepatitis as an explanation was unlikely. In the present study the patients' oxygen levels were continuously monitored and hypoxia was avoided.

The elevated transaminase levels in the majority of patients with underlying congenital heart disease (CHD) may have been on account of right ventricular failure causing ischaemic hepatitis due to hepatic venous congestion. This hypothesis is supported by a previously reported cohort study of adult patients with ischaemic hepatitis who had right ventricular failure as an underlying

cause in 94% of cases (Seeto, Fenn and Rockey 2000). Elevated AST levels have previously been reported in children with systemic venous congestion due to congenital heart disease (Mace, Borkat and Liebman 1985). Pulmonary hypertension, which is a well-described phenomenon in ventilated children with RSV bronchiolitis with CHD, may also have played a role (Moler et al. 1992; Khongphatthanayothin et al. 1999).

Elevated cTnT levels in patients with CHD may mean that cardiac failure, which has been described as a cause of elevated cTnT levels (Missov and Mair 1999), was exacerbated by severe RSV bronchiolitis. This could explain the significantly higher AST levels in patients with CHD compared to patients without CHD on the second and third day after admission (see Figure 3.2). At this time the strain to the right ventricle from pulmonary hypertension due to a peak in ventilation requirements may have been particularly severe. Another explanation for elevated cTnT levels may be RSV-associated myocarditis, which has been reported previously (Thomas et al. 1997; Huang, Bigos and Levine 1998).

In our study predicted probability of death calculated from PIM scores was not significantly higher in patients with elevated transaminase levels as compared to patients without elevated transaminase levels during course of disease. This may have been due to the fact that the PIM is calculated from data extracted on admission when eventual disease severity has not yet evolved. PIM is also an 'all-comers' score and not designed for specific diseases. Underlying congenital heart disease rather than the pathophysiology underlying transaminase level elevation may have accounted for the excess mortality in the group with elevated transaminase levels. In adults elevated transaminase levels have been identified as predictors of increased mortality in chronic cardiac failure and severe bacterial pneumonia requiring mechanical ventilation (Dahmash and Chowdhury 1994; Batin et al. 1995). Underlying CHD leading to right ventricular failure due to pulmonary hypertension, may have contributed to excess mortality and inotrope requirements in patients with CHD. Right ventricular failure associated with more severe pulmonary disease and leading to prolonged ventilation requirements, may also have been the

cause of temporarily elevated transaminase levels in patients without CHD.

Interpretation of the results is limited by the lack of a control group of similar children with severe RSV-negative bronchiolitis. Without such a comparable group it is difficult to assess whether the effects observed are a consequence of RSV infection or due to the severity of their disease per se. Future studies would be strengthened by a RSV-negative control group.

Whether hepatic transaminase level elevation, the magnitude of this elevation or cardiac troponin T levels in the first 3 days after PICU admission could be a useful predictor of mortality in children ventilated with severe RSV disease would require a specific larger, long-term prospective study in children with and without CHD.

3.5 *Conclusions*

Transaminase level elevation was common in children in our PICU ventilated with RSV bronchiolitis. There was no evidence that other common viral infections caused this phenomenon.

Disease severity as judged by duration of ventilation, inotrope use and mortality was greater in children with elevated transaminase levels (Table 3.1).

The greater severity of disease as judged by duration of ventilation was independent of the presence of CHD.

The elevated transaminase levels may have been a reflection of myocardial failure and/or ischaemic hepatitis secondary to myocardial failure.

4 Myocardial damage and disease severity in children with severe RSV bronchiolitis.

4.1 *Introduction*

Myocardial involvement during RSV bronchiolitis was first reported in 1972 in a case of fatal interstitial myocarditis in a child (Puchkov and Minkovich 1972). The reported degree of cardiac involvement during infection with this virus ranged from no detectable electrical or functional abnormality, focal involvement of the conducting system, supraventricular- and ventricular arrhythmias to global myocardial involvement with electrical and mechanical dysfunction (Giles and Gohd 1976; Menahem and Uren 1985; Menahem 1991; Hutchinson et al. 1994; Thomas et al. 1997; Huang, Bigos and Levine 1998). Cardiac arrhthmias have also been reported during RSV infection in patients with structurally normal hearts and were not associated with hypoxia or beta agonist therapy (Armstrong and Menahem 1993; Donnerstein et al. 1994; Thomas et al. 1997; Playfor and Khader 2005).

Cardiovascular compromise in the form of hypotension without cardiac arrhythmias has also been described and has been associated with evidence of myocardial damage as evident from elevated cardiac troponin I and T levels. An observational study of 218 infants admitted to a children's hospital with RSV bronchiolitis described 7 infants with features of shock (Njoku and Kliegman 1993). Elevated cardiac troponin levels have also been found in 35 – 54 % of infants ventilated on PICU with RSV bronchiolitis (Checchia et al. 2000). Additionally, elevated cardiac troponin I levels have also been found in children with RSV infection not requiring mechanical ventilation (Moynihan et al. 2003). The degree of cardiovascular support reported has ranged from administration of fluid boluses to inotropic support (Checchia et al. 2000; Kim and Frankel 1997; Moynihan et al. 2003).

RSV has been isolated from myocardial tissue in immunodeficient individuals (Fishaut, Tubergen and McIntosh 1980). A previous retrospective study on the prevalence of hepatitis associated with RSV-disease revealed that 13% of children admitted to our PICU with RSV bronchiolitis had significantly elevated aspartate aminotransferase (AST) levels (Eisenhut and Thorburn 2002). The elevated AST levels were suggestive of being of myocardial origin. All infants who had only elevated AST-levels died. Myocardial involvement may have contributed to these deaths from severe RSV disease.

Troponin is an inhibitory protein complex forming part of the contractile apparatus of all striated muscle, including cardiac muscle. Specific forms of the troponin subunits T, I and C exist in the various different muscle types. Cardiac troponin T (cTnT) is a myocardio-specific 37-kDa protein that is responsible for the binding of the myocardial troponin complex to tropomyosin. Cardiac TnT is a more sensitive and specific marker of myocardial damage than conventional cardiac enzyme levels (Immer et al. 1997; Lauer et al. 1997). Cardiac-specific troponins T and I have become established as the gold standard biochemical marler for myocardial necrosis and damage (Bhayana and Henderson 1995, Hetland and Dickstein 1998). Measurements of cTnT levels were therefore used to determine the prevalence of myocardial involvement (damage or injury) in infants admitted to PICU with severe RSV bronchiolitis. The relationship of cTnT levels to the clinical characteristics of the study patients, their myocardial abnormalities, indicators of disease severity and outcome was investigated.

4.2 *Patients and Methods*

4.2.1 Setting

20-bed regional multidisciplinary tertiary paediatric intensive care unit (PICU) in university-affiliated children's hospital.

4.2.2 Objectives

1. To determine the prevalence of myocardial involvement (damage, injury or strain) in severe RSV bronchiolitis as evident from elevated cardiac Troponin T (cTnT) levels.
2. To assess the nature of the myocardial involvement as manifested in electrocardiographic and echocardiographic abnormalities.
3. To compare severity of disease with and without myocardial involvement as evident from duration of ventilation, inotrope requirements and death.

4.2.3 Patients

Infants, both with and without congenital heart disease, admitted to the PICU during the winter season October 2002 to March 2003 with RSV bronchiolitis detected by RSV antigen in respiratory secretions (see 2.3.2.1 for details) were included in this prospective observational cohort study.

Written and informed consent by parents or guardians was obtained.

The Liverpool Children's Research Ethics Committee approved the study.

Exclusion criterion: Any infant that acquired their RSV in PICU (i.e. nosocomial RSV infection).

4.2.4 Measurements

4.2.4.1 Biochemistry:

Cardiac TnT levels were measured within 48 hours of PICU admission with a one-step monoclonal sandwich immunoassay (Roche Diagnostics Ltd) in the Department of Clinical Biochemistry at the Royal Liverpool Children's Hospital. Cardiac TnT levels were considered to be elevated if detectable (>10pg/ml). If cTnT levels were found to be elevated they were repeated until they became undetectable or until arterial access was lost or routine blood sampling ceased. Additionally see 2.3.2.3 for further details.

4.2.4.2 Cardiac:

Pulse wave Doppler echocardiographic assessment (using Vivid 5 or Vivid 7, General Electric Healthcare, Milwaukee, USA) was performed within the 48 – 72 hours of PICU admission by a Paediatric Cardiologist in enrolled patients - measurements included: left ventricular end diastolic and systolic dimension, and left ventricular fractional shortening. Measurements were repeated three times per patient and average values calculated. Two cardiologists reviewed and analysed the recordings independently. The average of their calculated values was utilized.

Electrocardiography (ECG) monitoring was routinely undertaken throughout the admission. Abnormalities on ECG monitoring were registered and formally recorded for cardiology analysis.

4.2.5 Analytic Methods:

Data were collected on included patients with a data extraction sheet, comprising of: age, gestational age at birth, year of admission, Paediatric Index of Mortality (PIM) score (Shann et al. 1997) on admission, history of chronic lung disease, congenital heart disease, history of neonatal intensive care, duration of symptoms before admission, duration of ventilation, inotrope use and its duration, death, cTnT levels, ECG- and echocardiographic findings.

Statistical analysis was performed with SPSS version 10.0 and Epi Info version 6.04b (CDC Atlanta). A result was considered statistically significant if $p < 0.05$.

4.3 *Results*

34 RSV-positive children were included in the study. Twelve children (35%) had elevated cTnT levels. The levels measured within 2 days of admission had a median (IQR) of 50 pg/ml (37.5 – 67.5pg/ml). Cardiac TnT levels decreased to undetectable levels at a median of 4 days after admission. In one patient with a haemodynamically significant ventricular septal defect the cTnT levels

reached a peak of 591pg/ml and were 40pg/ml when last measured 11 days after admission.

Data on baseline characteristics, clinical course and outcome are summarized in Table 4.1. Infants with elevated cTnT levels were generally younger than those with undetectable levels. Analysis of components of the PIM score revealed that there was no significant difference between patients with and without cTnT elevation with regards to FiO_2, PaO_2, ratio of FiO_2/PaO_2 and base excess on admission ($p > 0.05$). The mean (SD) systolic blood pressure (BP) was 73 (21) mmHg in children with and 95 (20) mmHg without elevated cTnT ($p = 0.01$). Four children in the group with elevated cTnT and none in the group with undetectable cTnT ($p = 0.01$) were found to be hypotensive with a BP more than 2 SD below the mean for age and sex (Task Force on Blood Pressure Control in Children 1987). None of these 4 infants had cardiopulmonary resuscitation or a hypoxic insult before admission. The duration of the hypotensive episode on admission had a median of 14.5 hours and management included repeated boluses of crystalloid and colloid fluids. Fractional shortening on echocardiograms performed at a median (IQR) of 3 days (2.5 – 4 days) after admission had a mean (SD) of 35% (8%) in subjects with elevated and 35% (6%) in patients with undetectable cTnT levels ($p = 0.94$).

Asymptomatic arrhythmias (one patient with supraventricular tachycardia and another with ventricular extrasystoles) on ECG were found in 2 cases with elevated cTnT levels and none of the patients with normal cTnT levels ($p = 0.12$).

Table 4.1: Demographics, clinical characteristics and course, and outcome of patients with RSV bronchiolitis with and without elevated cardiac troponin (cTnT) levels.

	cTnT elevated (n=12)	cTnT undetectable (n=22)	p-value
Male/female ratio	3.0	3.4	0.74[a]
Median age (IQR) in months	1.4 (0.7-2.0)	4.0 (1.7-6.6)	0.04[b]
Mean (SD) gestational age at birth	34.4 (3.7)	35.6 (5.1)	0.44[c]
History of neonatal intensive care	4	6	0.71[a]
History of chronic lung disease	0	3	0.53[a]
History of congenital heart disease	1	7	0.21[a]
Mean (SD) duration of symptoms before admission in days	3.4 (2.3)	4.1 (2.9)	0.50[c]
Mean (SD) predicted mortality according to PIM score on admission (%)	15.8 (4.3)	10.8 (2.1)	0.02[c]
Mean (SD) duration of ventilation in days	7.3 (8.0)	7.8 (6.7)	0.86[c]
High frequency ventilation used	0	3	0.25[a]
Inotrope use	0	1	1.0[a]
Death	0	1	1.0[a]

[a] Fishers exact test

[b] Mann Whitney U test

[c] Student's t-test.

4.4 *Discussion*

This study was the first to provide data on the prevalence of myocardial involvement in RSV lung disease. Myocardial involvement (injury or strain) was common and occurred in patients with structurally normal heart. It resolved in most cases within 4 days of admission. This makes myocarditis, in which a more persistent cTnT elevation would be expected, unlikely to be a cause. Cardiovascular function appeared to be transiently impaired in patients with elevated cTnT on admission as reflected by a low blood pressure at that time. Subsequent echocardiograms found no evidence of a persistent reduction in myocardial contractility. In previous reports cTnT elevation was found to be common in patients on PICU with systemic inflammatory response syndrome and it has been suggested that cytokines like tumour necrosis factor (TNF) are toxic to myocardial myocytes (Girior and Stromberg 2000). TNF release by the respiratory tract is a feature of the acute phase of RSV positive bronchiolitis (Matsuda et al. 1995). Cardiac TnT level elevation has been reported in patients with pulmonary disease - in preterm infants ventilated for respiratory distress syndrome and in two adults with chronic obstructive lung disease and lobar pneumonia (Trevisanuto et al. 2000; Weinberg, Cukierman and Chajek-Shaul 2002). Acute pulmonary hypertension associated with pulmonary embolism in adults without coronary artery disease is a well described cause of cTnT elevation and is thought to be due to myocardial ischemia (Giannitsis et al. 2000; Konstantinides et al. 2002). A prospective echocardiography study on infants with acute bronchiolitis found that features of pulmonary hypertension like pansystolic tricuspid regurgitation with high peak systolic velocity across the tricuspid valve were common and detectable in 75% of patients ventilated with acute bronchiolitis (Sreeram, Watson and Hunter 1991). The echocardiographic assessment in this specific study did not check for signs of pulmonary hypertension.

Elevated cTnT could be a reflection of the strain on the right ventricle associated with pulmonary hypertension associated with the poor gas exchange and lung compliance and hyperinflation of the lungs in the initial phase after admission to PICU. A reduction of myocardial strain by optimising oxygenation and reducing respiratory effort by mechanical ventilation and

sedation may have accounted for the rapid decline of cTnT levels. Cardiac TnT elevation can be cause or effect of hypotension. Healthy coronary arteries in infants make them less prone to myocardial ischaemia secondary to hypotension compared to adults. Hence it is more likely that the hypotension was consequence or associated with the myocardial insult causing the cTnT elevation. Systolic hypotension is a feature found in acute pulmonary hypertension (Giannitsis et al. 2000).

Although the patients with elevated cTnT levels were younger than those with undetectable cTnT levels, it is improbable that the cTnT elevation was related to a perinatal asphyxiating event, which has previously been described as being associated with elevated cTnT levels (Trevisanuto et al. 1998). Most patients with elevated cTnT levels were older than 3 weeks and cTnT has a half life in serum of 2 hours (Katus et al. 1991). Cardiac TnT has a short half life in serum of 2 hours (Katus et al. 1991), and therefore diminishes rapidly following an acute insult (e.g. perinatal asphyxia). Most of the study patients with elevated cTnT levels were older than 3 weeks. All the patients, both with and without elevated cTnT levels, had similar gestational ages at birth and histories of neonatal intensive care (Table 4.1). Cardiac TnT concentrations have previously not been found to be correlated to gestational age (Panteghini et al. 1997). Perhaps a younger myocardium copes less well with strain from severe parenchymal lung disease.

4.5 *Conclusions*

Raised cardiac troponin levels are common in infants with structurally normal hearts and severe RSV lung disease. This data may help to explain the increased morbidity and mortality associated with RSV bronchiolitis in children with significant congenital heart disease (Fixler 1996). Myocardial injury or strain, which, according to this data, occurs in infants with a structurally normal heart with severe RSV lung disease, may cause a heart already under strain from a structural abnormality to fail. However, whether the raised cTnT levels are specific to severe RSV lung disease per se or rather related to severe infective parenchymal lung disease in general remains to be determined.

5 Reduced right ventricular function in infants without congenital heart disease with severe RSV bronchiolitis.

5.1 *Introduction*

An extrapulmonary impact from RSV infection is well recognised (Eisenhut 2006). The first report of clinically symptomatic myocardial involvement during RSV bronchiolitis was that of a case of fatal interstitial myocarditis in 1972 (Puchkov and Minchovich 1972). The degree of cardiac involvement during RSV infection reported has ranged from electrical/conduction abnormalities (arrhythmias) to global myocardial involvement with mechanical dysfunction (Giles and Gohd 1976; Menahem and Uren 1985; Armstrong and Menahem 1993; Donnerstein et al. 1994; Thomas et al. 1997; Huang, Bigos and Levine 1998; Checchia et al. 2000; Eisenhut et al. 2004; Playfor and Khader 2005).

Previous studies investigating hypotension and inotrope requirements in infants ventilated with RSV bronchiolitis (Checchia et al. 2000; Eisenhut et al. 2004) demonstrated associated myocardial damage as evident from elevated cardiac troponin levels. Echocardiographic assessment of left ventricular ejection fraction revealed no difference between patients with and without elevated cardiac troponin T (cTnT) levels (Eisenhut et al. 2004) indicating no evidence of global or left ventricular failure. Findings suggested that right ventricular failure was the cause of elevated cTnT levels. Right ventricular strain resulting in ischaemic hepatitis due to hepatic venous congestion was also deemed to be a causative factor in studies that identified elevated transaminase levels in ventilated infants with RSV bronchiolitis, with the elevated transaminase levels being significantly more common in children with congenital heart disease (Eisenhut and Thorburn 2002; Eisenhut, Thorburn and Ahmed 2004). The above-mentioned studies (Checchia et al. 2000; Eisenhut and Thorburn 2002; Eisenhut et al. 2004; Eisenhut, Thorburn and

Ahmed 2004) therefore generated the following hypothesis: Right ventricular failure is associated with myocardial damage and causes ischaemic hepatitis in infants ventilated with RSV bronchiolitis.

5.2 *Patients and Methods*

5.2.1 Setting:

20-bed regional multidisciplinary tertiary paediatric intensive care unit (PICU) in university-affiliated children's hospital.

5.2.2 Objectives:

1. To establish whether severe RSV bronchiolitis is associated with reduced right ventricular function in infants without congenital heart disease.
2. To investigate the cause of right ventricular impairment by analysis of the relationship to left ventricular and atrial volumes and severity of respiratory disease.
3. To investigate whether reduced right ventricular function is associated with myocardial damage or strain.
4. To investigate whether reduced right ventricular function is associated with elevated transaminase levels.

5.2.3 Patients:

In a prospective observational study consecutive infants under 12 months of age and ventilated with severe RSV bronchiolitis, confirmed on RSV antigen testing, immunofluorescence and/or culture, were enrolled after informed written consent by parents or carers. The study was approved by the Liverpool Children's Research Ethics Review Committee.

Exclusion criteria: Any child that acquired their RSV in PICU (i.e. nosocomial RSV infection). Children with evidence of congenital heart disease were

excluded in order to avoid underlying myocardial disease confounding interpretation of data.

5.2.4 Measurements:

5.2.4.1 Virology

Nasopharyngeal aspirates were tested by the Directigen^{TM} RSV test [Becton Dickinson microbiology systems, Maryland, USA]. These are *in vitro* immunochromatographic assays for the rapid and qualitative detection of RSV antigen directly from nasopharyngeal specimens. Samples negative for RSV using the rapid antigen tests were cultured using standard virological techniques at the Health Protection Agency (Aintree, Liverpool, UK). Immunofluorescence was performed using standard virological techniques at the Health Protection Agency.

5.2.4.2 Biochemistry:

Cardiac troponin T (cTnT) levels were determined, as a marker of myocardial damage (17Immer et al 1997), on the day of echocardiographic assessment with a one-step monoclonal sandwich immunoassay (Roche Diagnostics Ltd) in the Department of Clinical Biochemistry at the Royal Liverpool Children's Hospital. Elevated levels were defined as values of >10pg/ml. Urea and creatinine levels, transaminase levels (alanine and aspartate aminotransferase levels), and C-reactive protein (CRP) levels were determined daily. Normal ranges for urea was 2.3 - 6.4 mmol/l and for creatinine 24 - 52 umol/l. Normal ranges for alanine aminotransferase was 9-36 IU/l and for aspartate aminotransferase 15-58 IU/l (Hicks and Soldin 1995). An elevated C-reactive protein level was defined as a level of > 10mg/l.

5.2.4.3 Cardiac:

Pulse wave Doppler echocardiographic assessment (using Vivid 5 or Vivid 7, General Electric Healthcare, Milwaukee, USA) was performed by a Paediatric

Cardiologist (SA, SN) on admission in all enrolled patients - measurements included: pulmonary valve ejection time, tricuspid valve closure time, left ventricular end diastolic and systolic dimension and left ventricular fractional shortening. Measurements were repeated three times per patient and average values calculated. The two cardiologists (SA, SN) reviewed and analysed the recordings independently. The average of their calculated values was utilized. The right ventricular Tei index was calculated as described previously (Tei et al. 1996) from tricuspid valve closure time (time interval between cessation and onset of tricuspid inflow) (a) and pulmonary valve ejection time (b) following the formula: Right ventricular Tei index = a-b/b. This is a reliable and reproducible technique as demonstrated in a previous study that revealed inter-observer and intra-observer coefficients of variation of 3.3 and 4.5% respectively (Tei et al. 1996). An elevated Tei index indicating reduced right ventricular function was defined as an index of more than 2 standard deviations (SD) above the mean for children (across all age groups and both genders) of 0.24 (SD 0.04) (Ishii et al. 2000).

Electrocardiography (ECG) monitoring was routinely undertaken throughout the admission with any abnormalities leading to formal tracing and analysis.

5.2.5 Analytic Methods:

Data were collected on a standardised data collection form including: age, gender, ventilation and oxygenation indices (Peters et al. 1998), duration of mechanical ventilation, calculation of the right ventricular Tei index, left ventricular fractional shortening and dimensions, cardiac troponin T levels, transaminase and C-reactive protein levels during stay on the PICU. Non-parametric data analysis using median and range, Mann-Whitney test for comparison of continuous data, and Fishers' exact test for categorical data were used because of small and unequal sample size. A p-value of <0.05 was considered to indicate statistical significance of difference. All p-values were two tailed. Software used for analysis was SSPS version 15.0 and Epi-Info version 6.04 (CDC, Atlanta).

5.3 *Results*

Thirty four ventilated infants with confirmed RSV bronchiolitis and no underlying congenital heart disease were enrolled over two RSV seasons (2003 – 2005). Median age was 1.4 months [range 0.4 – 11.7] and median length of ventilation 5 days [range 2 – 10].

Echocardiography was performed within 24 hours of PICU admission in 79% (27/34) of the cases and within 48 hours for the remainder. Seven (20%) infants had an elevated right ventricular Tei index indicating significantly reduced right ventricular function - median Tei index 0.46 [range 0.33 – 0.92]. The group with a normal Tei index had a median of 0.19 [range -0.17 – 0.31] ($p < 0.01$). Age, gender, left ventricular function including fractional shortening, left ventricular end diastolic and systolic diameters, left atrial diameter and admission systolic blood pressure were not different comparing patients with and without right ventricular dysfunction (Table 5.1). None of the patients in this study required inotropes. Tricuspid regurgitation was found in eight patients (24%) - 2/7 with an elevated Tei index [calculated pulmonary artery pressure range 25 – 35mmHg] and 6/27 [calculated pulmonary artery pressure range 25 – 35mmHg] with a normal Tei index ($p = 0.8$). Three patients (3/7) with elevated and eleven with normal Tei index (11/27) had a detectable cardiac troponin T level ($p = 1.0$). Cardiac troponin T (cTnT) was therefore raised in 14 (40%) of the 34 ventilated children with RSV bronchiolitis - median measurement 30 ng/l [range 10 – 140]. There was no difference in age of patients with (median 0.9 months, range 0.4 – 11.7) and without elevated cTnT levels (median 1.45 months, range 0.4 – 6.4) ($p = 0.32$). There was no difference in left ventricular (fractional shortening) and right ventricular function (Tei index) in infants with elevated cTnT (median fractional shortening 32% [range 21 – 39] and median Tei index 0.22 [range -0.17 – 0.55]) and without elevated cTnT (median fractional shortening 32% [range 16 – 45] and median Tei index 0.22 [range 0.14 – 0.92]) levels ($p = 0.70$ and $p = 0.75$ respectively).

No arrhythmias or other ECG abnormalities were observed in either group.

Table 5.1: Echocardiographic data in children ventilated with respiratory syncytial virus infection with and without right ventricular dysfunction as demonstrated by an elevated or 'normal' Tei-index (Tei et al. 1996).

	Elevated Tei index (n=7)	Normal Tei index (n=27)	p-value*
Gender (male)	4	17	1.0
Age (months, median, [range])	1.1 [0.4-3.3]	1.5 [0.4-11.7]	0.56
Left ventricular fractional shortening (%, median, range)	32.6 [22-45]	32.1 [16-42]	0.45
Left ventricular end diastolic diameter (cm, median, range)	1.8 [1.4-2.1]	1.8 [1.4-2.6]	0.45
Left ventricular end systolic diameter (cm, median, range)	1.1 [1.0-1.3]	1.3 [0.9-1.8]	0.06
Left atrial diameter (cm, median, range)	0.83 [0.57-1.0]	0.90 [0.61-1.47]	0.27
Admission systolic blood pressure (mmHg, median, range)	65 [62-86]	75 [42-120]	0.18

* Mann-Whitney test

Alanine aminotransferase levels were elevated in 3/7 and aspartate aminotransferase levels in 2/7 patients with elevated Tei index and in 12/27 patients each with normal Tei index ($p = 0.21$ and 0.67 respectively)

Highest transaminase and C-reactive protein levels, highest ventilation and oxygenation indices, as well as duration of mechanical ventilation, were not different between patients with and without elevated Tei index (Table 5.2).

Table 5.2: Clinical and laboratory parameters in children ventilated with respiratory syncytial virus infection with and without right ventricular dysfunction as demonstrated by an elevated or 'normal' Tei-index (Tei et al. 1996).

	Elevated Tei index (n=7)	Normal Tei index (n=27)	p-value
Admission ventilation index (median, range)	27.2 [21.0-34.4]	23.4 [16.9-29.3]	0.77*
Admission oxygenation index (median, range)	6.0 [4.1-9.5]	5.9 [4.8-11.4]	0.60*
Highest ventilation index (median, range)	28.1 [24.2-65.2]	27.8 [14.9-80.2]	0.43*
Highest oxygenation index (median, range)	8.1 [3.7-18.6]	5.9 [1.5-19.5]	0.59*
Duration of mechanical ventilation (days, median, range)	5.0 [4.0-7.0]	5.0 [2.0-10.0]	0.62*
Elevated cardiac troponin T level (>10 ng/l)	3	11	1.00†
Highest ALT (IU/l, median, range)[a]	35 [21-379]	28 [13.0-339]	0.21*
Highest AST (IU/l, median, range)[a]	54 [25-103]	46 [25-126]	0.78*
Highest C-reactive protein level (mg/l, median, range)[a]	26 [8-142]	41 [7-179]	0.58*

* Mann-Whitney test, † Fisher's exact test

[a] Highest level during stay on the Paediatric Intensive Care Unit

ALT = alanine aminotransferase level AST = aspartate aminotransferase level

5.4 *Discussion*

Significantly reduced right ventricular function was found in 20% of infants without congenital heart disease ventilated for RSV bronchiolitis. This reduced right ventricular function was not related to the severity of respiratory disease. This finding in infants with severe RSV disease is contrary to an earlier study of 10 infants with RSV disease which did not find an increased right ventricular ejection time and right ventricular systolic time intervals in patients with moderate to severe compared to patients with mild disease (Pahl and Gidding 1988). Unlike the present study the authors concluded that RSV bronchiolitis was not associated with a significant depression of cardiac performance (Pahl and Gidding 1988).

This study is the first employing a standardised right ventricular performance index in infants with RSV disease and the largest containing data on right ventricular performance in severe RSV infection requiring ventilation published so far. The Tei index was utilized as it is non-invasive, uncomplicated, reproducible, and not significantly influenced by heart rate, right ventricular pressure, the ventricular dilatation or, importantly, tricuspid regurgitation (Tei et al. 1996; Ishii et al. 2000). Because the index is essentially a time ratio it is not affected by ventricular geometry and is useful in assessment of global right ventricular function in children with their complex right ventricular shape (Eiden et al 1998; Tsutsumi et al. 1999; Ishii et al. 2000). The index has been shown to correlate well with clinical symptoms of right ventricular failure and overall survival (Eiden et al 1998; Tsutsumi et al. 1999; Ishii et al. 2000; Burgess, Bright-Thomas and Ray 2002). A normal range in healthy children has been established (Tei et al. 1996; Ishii et al. 2000).

As with prior studies, raised cardiac troponin levels (cTnT) were found commonly in children with RSV infection – present study 41% of cases; Checchia et al. 54.5% (Checchia et al. 2000); Moynihan et al. 40% (Moynihan et al. 2003); Eisenhut et al. 35% (Eisenhut et al. 2004). In an earlier study in our PICU, cardiovascular function appeared to be transiently impaired in

children with elevated cTnT on admission as reflected by a low blood pressure at that time (Eisenhut et al. 2004). Echocardiographic assessment of left ventricular function found no evidence of reduction in myocardial contractility, but right ventricular function was not specifically assessed (Eisenhut et al. 2004). In this study, despite raised cardiac troponin levels, any global or left ventricular dysfunction clinically or on echocardiography could not be demonstrated. Contrary to the primary hypothesis, raised cardiac troponin / myocardial damage was not related to right ventricular dysfunction. A feature further dispelling the hypothesis was the finding of a normal cTnT level in a child with severe right ventricular dysfunction according to the categorisation of Eiden et al (Eiden et al. 1998).

The original hypothesis was further eroded by the finding that transaminase levels were not higher in patients with right ventricular dysfunction. It does not support the concept of right ventricular strain resulting in hepatic venous congestion leading to elevated transaminase levels in ventilated infants with RSV bronchiolitis. It is conceivable that children with RSV bronchiolitis and congenital heart disease, excluded from this study, may have more severe and chronic right ventricular failure leading to transaminase level elevation from hepatic venous congestion and ischaemic hepatitis (Eisenhut and Thorburn 2002). The inflammatory response to RSV reflected in CRP levels was not more marked in patients with right ventricular dysfunction. Potentially cardio-depressant inflammatory markers (e.g. tumour necrosis factor, interleukin-6, etc.) were not measured and future studies are required to assess their correlation with right ventricular dysfunction in children with RSV bronchiolitis.

Echocardiographic determination of pulmonary artery pressures in a normal heart is usually reliant on tricuspid regurgitation to generate Doppler-derived measurements to compare to systemic arterial pressure. Tricuspid regurgitation was not a common finding in our study population and therefore other indirect measurements of pulmonary artery pressure (e.g. left ventricular and left atrial dimensions) were utilized. Left ventricular and atrial dimensions, which are reduced in significant pulmonary hypertension (Patel et al. 1980)

were not different comparing patients with and without right ventricular dysfunction in the reported study. Additionally, there was no evidence of volume overload, which causes a reduction of left ventricular ejection fraction (EF) (Patel et al. 1980), as fractional shortening was not different in patients with and without right ventricular dysfunction. Tei index has been shown to reflect right ventricular failure in adult patients with primary pulmonary hypertension (Tei et al. 1996; Yeo et al. 1998). In children with an atrioseptal defect (ASD) without pulmonary hypertension, the Tei index was normal (Ishii et al. 2000). Despite suspicions, significant pulmonary hypertension, which has been associated with severe RSV bronchiolitis in previous studies (Sreeram, Watson and Hunter 1990; Fitzgerald et al. 2001), could not be clearly demonstrate as a cause of right ventricular dysfunction. Without tricuspid regurgitation, all that can be reported with any confidence is that the pulmonary artery pressures were not systemic or suprasystemic, but not whether the pulmonary artery pressures were higher than normal.

Although right ventricular dysfunction has been clearly demonstrated in the study group, the cause for the right ventricular dysfunction is less forthcoming. I suspect that the small sample size, especially in the group with an elevated Tei index, has limited the ability to expose trends or aetiology in this patient group. Ventilation with a high positive end expiratory pressure strategy has previously been associated with right ventricular strain (Dhainaut and Brunet 1990), but that was not a concerted approach in this study group and therefore unlikely to have differentiated the two subgroups. Even though right ventricular preload can be influenced by the degree of lung expansion or overinflation, this was not different in the two groups as reflected by similar ventilation and oxygenation indices. A limitation of this study is the lack of a comparative non-RSV infected group to help differentiate whether it is RSV itself that causes right ventricular impairment. The immunological process triggered by the cellular immune response against RSV may cause collateral immune-mediated damage to myocardial tissue and hepatocytes (Eisenhut 2006). Direct invasion of the myocardium and liver has been identified and may cause myocarditis or hepatitis (Eisenhut 2006). However a raised cardiac

troponin would be expected with RSV myocarditis. RSV-induced hepatitis as a possible cause for raised transaminases was investigated in previous studies in which no other known hepatitis viruses or respiratory viruses known to cause hepatitis was found in a similar patient group (Eisenhut and Thorburn 2002; Eisenhut, Thorburn and Ahmed 2004). A liver biopsy looking for hepatic RSV infection would make or disprove such a diagnosis, but is inappropriate on ethical grounds. Future larger studies need to corroborate the findings of this study particularly with simultaneous measurement of pulmonary artery pressures to verify whether pulmonary artery hypertension impacted on right ventricular function / dysfunction. Once causative factors of reduced right ventricular function are identified the next step would be to assess the influence of these factors in infants with congenital heart disease infected with RSV, as this subgroup have increased morbidity and mortality from severe RSV disease (Howard et al. 2000; Holman et al. 2003; Panickar et al. 2005).

5.5 *Conclusions*

Right ventricular dysfunction is not uncommon in severe RSV disease, but the degree of dysfunction is not related to the levels of respiratory support or biochemical markers of inflammation.

Myocardial and hepatocellular damage occurs in infants ventilated with RSV bronchiolitis with normal right and left ventricular function.

6 Pulmonary bacterial co-infection in children with severe RSV bronchiolitis.

6.1 *Introduction*

Respiratory syncytial virus (RSV) is the most important viral cause for lower respiratory infection in infants and young children throughout the world (Howard et al. 2000). It is one of the commonest causes of respiratory tract infection leading to respiratory failure. It has been estimated that in each year 600,000 deaths occur world-wide that are directly or indirectly attributable to RSV (Howard et al 2000). Factors that increase susceptibility to the virus include chronological age less than 6 weeks, bronchopulmonary dysplasia, congenital heart disease, prematurity, and immunodeficiency (MacDonald et al. 1982; Navas et al. 1992; Langley et al. 1997a; Buckingham et al. 2001). Although the mortality rate for those hospitalized may be as low as 1 – 3%, the mortality rate increases in those with severe bronchiolitis requiring intensive care management . (MacDonald et al. 1982; Navas et al. 1992; Langley et al. 1997a+b; Buckingham et al. 2001; Thorburn et al. 2004). In developed countries about 2% of infants and children admitted to hospital with RSV require assisted ventilation (Behrendt et al. 1998). RSV bronchiolitis is a common cause for admission to a PICU in the winter season (Behrendt et al 1998; Buckingham et al. 2001; Thorburn et al. 2004).

The pharmacological management of RSV bronchiolitis, other than the use of supplementary oxygen, has long been debated (Simoes 1999; Harrison et al. 2001). In particular, many advocate against the routine use of antibiotics in bronchiolitis on account of a reported low incidence of concurrent or secondary bacterial infections in RSV patients (Friis et al. 1984; Hall et al. 1988; Korppi et al. 1989; Todd 1990; Kuppermann et al. 1997; Purcell and Fergie 2002; Titus and Wright 2003; Bloomfield et al. 2004; Levine et al. 2004). However, these studies focused on extrapulmonary bacterial co-infection and included only limited numbers of children with severe respiratory compromise/failure.

Physiologically the lower airways are normally sterile. Nevertheless, the relationship of bacterial co-infection with viral respiratory disease has been recognised previously, with an escalating incidence with increasing severity of respiratory illness (Hament et al. 1999). Three retrospective studies investigated the occurrence of bacterial co-infection in children with severe RSV infection requiring PICU admission and found the incidence of pulmonary bacterial co-infection to vary between 17.5% - 44% (Duttweiler, Nadal and Frey 2004; Randolph, Reder and Englund 2004; Kneyber et al. 2005).

In this study the incidence of pulmonary bacterial co-infection using established quantitative microbiology (American Thoracic Society 2005) in patients with severe RSV bronchiolitis on admission to a tertiary PICU was prospectively investigated and the impact of the bacterial co-infection on morbidity and mortality evaluated.

6.2 *Patients and Methods*

6.2.1 Setting:

20-bed regional multidisciplinary tertiary paediatric intensive care unit (PICU) in university-affiliated children's hospital.

6.2.2 Objectives:

1. To determine the incidence of pulmonary bacterial co-infection in patients requiring PICU for severe RSV bronchiolitis.
2. To study the impact of the co-infection on morbidity, including length of ventilation and inflammation, and mortality.

6.2.3 Patients:

All children with severe RSV bronchiolitis, confirmed on RSV antigen testing and/or culture, requiring PICU and on whom lower airway secretions were obtained on admission were studied prospectively during 3 consecutive RSV seasons (winter seasons) between 2002 and 2005.

The Liverpool Children's Research Ethics Committee waived the need for formal ethical approval as the study fell into the realms of clinical audit.

Exclusion criteria: Nosocomial cases of RSV infection in the PICU.

6.2.4 Respiratory support:

Intubation was performed by our PICU retrieval team at the referring hospital, in our accident & emergency (A&E) department, or in one of the hospital wards prior to PICU admission. Alternatively, the anaesthetic team of the referring hospital intubated some of the patients preceding the arrival of our PICU retrieval team. It is policy that all children who require intensive care and ventilatory support are moved to the regional PICU.

The timing of extubation was judged clinically and not influenced by bronco-alveolar lavage (BAL) results.

6.2.5 Microbiologic sampling:

Diagnostic samples of nasopharyngeal aspirates (for RSV detection) and lower airway secretions (for bacterial culture) through endotracheal tube using sterile precautions (de Blic et al. 2000) were taken on admission and processed immediately in the laboratory. Prior to routine bronchial toilet a sterile suction catheter was passed down the endotracheal tube. Two 1ml/kg aliquots of sterile 0.9% saline were instilled through the suction catheter, immediately followed by aspiration with constant pressure into a mucus trap. Samples were collected by specialist respiratory physiotherapists or PICU staff members. BAL was performed immediately after endotracheal intubation in children intubated in our hospital and on arrival in the PICU - generally within three hours of endotracheal intubation for those retrieved from other hospitals.

All children within the region are only ventilated in the regional PICU, so are rapidly transferred to the PICU.

Surveillance samples of throat and rectum were obtained on admission and then twice weekly, in keeping with the routine surveillance practice in our unit.

6.2.6 Measurements:

6.2.6.1 Virology:

Nasopharyngeal aspirates were tested by the Directigen^{TM} RSV test [Becton Dickinson microbiology systems, Maryland, USA]. This is an *in vitro* enzyme immunoassay [ELISA] membrane test for the rapid and qualitative detection of RSV antigen directly from nasopharyngeal specimens. All samples negative for RSV using the ELISA membrane test were cultured using standard virological techniques at the Public Health Laboratory. See 2.3.2.1 for further details.

6.2.6.2 Bacterial/Yeast

Diagnostic or clinical samples were processed immediately in a qualitative and semi-quantitative way using standard microbiological methods. For all types of samples, macroscopically distinct colonies were isolated in pure culture. Broncho-alveolar lavages were centrifuged at 1200xg for 10 minutes. All but 0.5ml of the supernatant was removed and the centrifuged deposit re-suspended in the remaining fluid. A sterile calibrated loop was used to plate out 0.01ml of the BAL sample. Thereafter the agar plates were incubated. Less than 10 colonies on the agar plate equated to $<10^3$ CFU/ml; between 10-100 colonies equated to 10^3-10^4 CFU/ml; >100 colonies to $>10^4$ CFU/ml; and >1000 colonies to > 10^5 CFU/ml of BAL. Chocolate, MacConkey, mannitol salt, and blood agars were used for bacterial isolation; and Sabuoraud agar for fungal isolation.

Standard methods for identification, typing, and sensitivity patterns were used for all micro-organisms (Brook 1979, Baselski and Wunderlink 1994, Standards Unit HPA UK 2009).

6.2.6.3 Chest radiology

Chest Xray appearance was not utilized to diagnose bacterial co-infection as chest Xray changes are not pathognomonic of secondary bacterial or viral infections (Friis et al 1990; Hament et al. 1999).

6.2.7 Antibiotic policies

Patients with signs of infection received intravenous cefotaxime (150 mg/kg/day 4 times daily for up to 7 days) as first line therapy for 48 hours whilst awaiting culture results. Clinical status on presentation governed whether supplementary intravenous cover with an aminoglycoside, gentamicin (7.5 mg/kg/day 3 times daily for up to 7 days) was added. Antibiotics were rationalized once culture and sensitivity results became available.

6.2.8 Definitions:

Bacteria positive - The presence of micro-organisms in the lower airways which is normally sterile.

Co-infection - Infection is a microbiologically proven, clinical diagnosis of inflammation, local and/or generalized. In this study clinical signs were unreliable as all patients had bronchiolitis, so microbiological definitions were utilized. Bacterial co-infection required bacteria colony counts $\geq 10^5$ CFU/ml of diagnostic sample for each single species obtained from lower airway secretions and, on a semi-quantitative scale of + = few (<50 X 10^6/L), ++ = moderate (>100 X 10^6/L) and +++ = many leucocytes (>1000 X 10^6/L), the presence of at least a moderate [++] number of leucocytes (A'Court et al. 1993; Sarginson et al. 2001; van Saene, Damjanovic and Alcock 2001).

Low bacterial growth - Diagnostic samples from lower airway secretions which yielded $<10^5$ CFU/ml of diagnostic sample and only a few [+] leucocytes.

6.2.9 Analytic Methods:

Data were collected prospectively. Prediction of mortality using paediatric index of mortality (PIM) was obtained on the patient's first contact with the PICU team (Shann et al 1997). Results were expressed as a percentage of the total study population; median and inter-quartile ranges (IQR) were used to describe the demographic distributions.

Continuous data was analyzed using the Wilcoxon-Mann-Whitney (W-M-W) test. Categorical data was analyzed using Fisher's exact or McNemar's test. Correlation was assessed using Spearman's rank test (two-tailed). Multivariate analysis was performed using linear and logistic regression analysis.

Statistical calculations were performed with the Statistical Program for Social Science release 11.0.0 (SPSS 11, Chicago, IL). A p value < 0.05 was considered statistically significant.

6.3 *Results*

A total of 181 children were admitted to the PICU with RSV positive bronchiolitis during the three consecutive RSV seasons (2002 – 2005). The indication for PICU admission for these children was ventilatory/respiratory support (respiratory failure (172) and/or life-threatening apnoeas (9)). All patients were mechanically ventilated for a median of 5.0 days [IQR 3.0 – 7.3]. Median age was 1.6 months [IQR 0.5 – 4.6]. 103 were boys and 78 girls. 165 children were enrolled in the study – an admission bronco-alveolar lavage (BAL) sample was not available in 16 patients (8.8%).

Table 6.1 shows the demographics, inflammatory marker values, antibiotic history and mortality of the RSV-positive critically ill children in the subgroups: RSV only, bacterial co-infection, low bacterial growth and bacteria positive (co-infection + low bacterial growth). Admission white cell count, neutrophil count and CRP did not differentiate between the groups, and neither did these indices during PICU days 1 – 5.

Although all patients were admitted primarily for respiratory disease, 43% (71/165) of them had other co-morbidities – congenital heart disease 37, chronic lung disease 8, immunodeficiencies 4, abnormality of large airways 5, congenital heart disease and abnormality of large airways 8, congenital heart disease and chronic lung disease 4, neuromuscular disease 7. Co-morbidity did not increase the risk of positive bacterial cultures (odds ratio = 0.77, 95% CI 0.55 to 1.09).

Overall 45% (74/165) received antibiotics prior to PICU admission (i.e. started by the referring hospital or ward), most often cefotaxime or ceftriaxone. The breakdown between the subgroups is shown in Table 6.1. Receipt of antibiotics prior to PICU admission did not affect PIM (W-M-W test p = 0.6) and LOV (W-M-W test p = 0.2). All patients, bar eight, were continued or commenced on antibiotics in the PICU – usually cefotaxime. Antibiotics were continued for a median of 5 days [IQR: 3 – 6 days]. The empiric use of antibiotics was at the discretion of the attending consultant. Gender, age groups, PIM, co-morbidity, receipt of prior antibiotics, time on antibiotics prior to intubation, admission oxygen and ventilation index were not predictive of positive bacterial cultures by uni- or multivariate analysis (all p-values > 0.16).

The organisms that were isolated from lower airway secretions obtained on admission are shown in Table 6.2.

All those with positive endotracheal bacteriology, had the same organisms isolated on admission surveillance swabs. Community organisms accounted for 83% (81/98) of the bacteria cultured.

Table 6.1: Patient characteristics according to broncho-alveolar culture result – median [interquartile range].

	RSV only	**Bacterial co-infection** ($> 10^5$ CFU/ml)	**Low bacterial growth** ($< 10^5$ CFU/ml)	**Bacteria positive** (Co-infection + low bacterial growth)
Number (total 165) and **percentage of total number**	**95** **57.6%**	**36** **21.8%**	**34** **20.6%**	**70** **42.4%**
Origin (Retrieved/intra-hospital/A&E)	**48/29/18**	**11/17/8**	**14/11/9**	**25/28/17**
Age (months)	**1.4** [0.4 – 3.9]	**1.3** [0.7 – 2.5] p = 0.8*	**3.5** [1.2 – 10] p = 0.04†	**1.8** [0.9 – 4.6] p = 0.9‡
Paediatric Index of Mortality (PIM)	**0.08** [0.03 – 0.12]	**0.09** [0.04 – 0.14] p = 0.6*	**0.08** [0.06 – 0.12] p = 0.5†	**0.08** [0.05 – 0.13] p = 0.6‡
Length of ventilation (days)	**4** [3 – 7]	**6** [4 – 8] p < 0.01*	**6** [5 – 9] p < 0.01†	**6** [4 – 8] p < 0.01‡
Admission Oxygen Index (OI) in the PICU	**8** [5 – 12]	**6** [4 – 9] p = 0.2*	**9** [6 – 12] p = 0.6†	**7** [4 – 11] p = 0.7‡
Admission Ventilation Index (VI) in the PICU	**26** [18 – 39]	**27** [16 – 44] p = 0.9*	**26** [20 – 32] p = 0.2†	**27** [19 – 39] p = 0.8‡
White Cell Count (X 10^9 cells/l) on PICU admission	**9.8** [7.2 – 13.7]	**10.6** [7.1 – 13.5] p = 0.5*	**11.5** [6.9 – 14.7] p = 0.6†	**11.3** [7.1 – 13.8] p = 0.4‡
Neutrophil count (X 10^9 cells/l) on PICU admission	**5.2** [2.9 – 7.6]	**7.1** [3.9 – 10.3] p = 0.1*	**5.8** [3 – 10.3] p = 0.9†	**6.2** [3.6 – 10.3] p = 0.2‡
C-reactive protein / CRP (mg/l) on PICU admission	**14** [4 – 45]	**14** [5 – 52] p = 0.9*	**21** [4 – 46] p = 0.9†	**18** [4 – 49] 0.9‡
Antibiotics prior to PICU admission	48%	36%	44%	40% p = 0.08‡
Time on prior antibiotics (days)	1 [1 – 2]	1 [1 – 3]	1 [1 – 3]	1 [1 – 3]
Mortality: (RSV-related deaths)	**8** (3)	**2** (1)	**2** (1)	**4** (2)
Percentage with co-morbidities§	40%	61%	41%	51% p = 0.6‡

CFU/ml = colony forming units of a single bacterial species per ml of diagnostic sample
Retrieved = patients retrieved from other hospitals
Intra-hospital = patients admitted from wards with our hospital
A&E = patients admitted directly from the Accident & Emergency department

* RSV-only vs. Bacterial co-infection † RSV-only vs. Low bacteria growth
‡ RSV-only vs. All those positive for bacteria (bacterial co-infection + low bacteria growth)
Wilcoxon-Mann-Whitney test used, except for prior antibiotics and co-morbidities – McNemar's test.

§ Co-morbidities = congenital heart, chronic lung disease, abnormality of large airways, immunodeficiencies, neuromuscular disease

OI = mean airways pressure (MAP) x FiO_2/PaO_2
VI = respiratory rate x $PaCO_2$ x peak inspiratory pressure / 1000

Table 6.2: The 98 bacterial isolates obtained on admission to PICU from the lower airway in 70 children with severe RSV bronchiolitis.

	Co-infection ($> 10^5$ CFU/ml)	**Low bacterial growth** ($< 10^5$ CFU/ml)
Community organisms[28,30]		
H. influenzae	17	11
S. aureus	10	12
M. catarrhalis	8	10
S. pneumoniae	6	6
S. pyogenes	1	
Abnormal organisms[28,30] ¶		
P. aeruginosa	4	3
B. pertussis	1	
K. pneumoniae	1	1
E. coli	1	1
E. cloacae and *C. freundii*	1	
P. mirabilis		1
S. agalactiae		1
N. meningitidis		1
MRSA		1

23 patients had multiple organisms (18 had two, 5 had three bacteria) – Community organisms were involved in 91% of these cases, compared to 77% of single isolates. (Fisher's exact p = 0.2)
¶ 67% (10/15) had chronic illnesses

There were twelve deaths (6.6%), of which 5 appeared to be RSV-related (2.8%) as the patients were still RSV positive when they died. Two were oncology patients (leukaemics on chemotherapy) that died from RSV pneumonitis on day 1 and 16 respectively. Neither had proven bacterial co-infection and both received broad spectrum empiric antibiotic therapy.

Other associated causes included singles cases of: hypoplastic right heart coupled with cystic fibrosis (on day 8), *Bordetella pertussis* co-infection with hypoxaemic respiratory failure requiring extracorporeal membrane oxygenation (ECMO) (on day 26), and a child with a congenital myopathy (on day 8). The remaining 7 deaths occurred 6 – 31 days after admission subsequent to the RSV cultures becoming negative. Causes of these RSV 'un-related' deaths

included: complex congenital heart disease 3, multiple congenital anomalies 2, congenital myopathy 1, anoxic brain injury 1.

Positive bacterial cultures did not predict death (odds ratio = 1.3, 95% CI 0.57 to 2.95), but co-morbidity did (odds ratio = 0.51, 95% CI 0.37 to 0.7).

6.4 *Discussion*

This observational study over 3 consecutive RSV seasons evaluating bacterial pulmonary co-infection found that 42% of children admitted for severe RSV infection harboured bacterial pathogens in their lower airways. These critically ill children run a serious risk of developing bacterial pneumonia (Hament et al. 1999; Liberati et al. 2004; Liberati et al. 2006).

BAL samples were collected very soon after intubation so significant growth densities of bacteria reflect pathogens in the normally sterile lower airways. The high number of colony forming units makes it highly unlikely that the micro-organisms isolated were 'pushed down' the trachea on intubation. It is acknowledged that the number of leukocytes in lower airways secretions will also be influenced by RSV infection and therefore bacterial growth was primarily utilised. The microbiological criteria were strict and avoided potentially confounding clinical factors. This microbiological approach is supported by recent literature concerning ventilator-associated pneumonia (VAP) (Gauvin et al. 2002; Gauvin et al. 2003). On the other hand, it must be appreciated that the study group was very different from this VAP group, as they had 'virgin', iatrogenically uncontaminated lower airways. Certainly the group defined as co-infection had substantial bacterial growth densities far too soon after intubation to have been oropharyngeal flora transported there by the endotracheal tube. If anything, the strict microbiological criteria probably underestimated the number co-infected by categorizing many of them as low bacterial growth. It should be appreciated that differentiating the groups into co-infected and low bacterial growth may be somewhat artificial as the lower respiratory tract should be free from bacteria.

The term "co-infection" was used, as, at the time of PICU admission, these infections could either be secondary or concurrent. It would not be easy to detect the 'chicken' from the 'egg' as far as which was primary, the RSV or the bacteria, although a viral infection destroying cilia is in general required for a bacterial co-infection (Hament et al. 1999). The true co-infection rate is likely to be higher than the 22% rate detected, as 45% of the cases received antibiotics prior to PICU admission. These antecedent antibiotics may have converted some of the "co-infection" patients into the "low bacterial growth" group, or even prevented bacterial growth altogether.

Previous studies have looked at bloodstream, otitis media or urinary tract infections in children with bronchiolitis, very few of whom had severe RSV bronchiolitis requiring intensive care (Friis et al. 1984; Hall et al. 1988; Korppi et al. 1989; Todd 1990; Kuppermann et al. 1997; Purcell and Fergie 2002; Titus and Wright 2003; Bloomfield et al 2004). These studies generally found a very low incidence of secondary serious bacterial infection (1.2%) (Hall et al. 1988) or bacteraemia (0.6%) (Bloomfield et al. 2004) in their hospitalized RSV patients. Because these studies did not specifically concentrate on those with severe bronchiolitis, it is difficult to extrapolate their results to this population. Duttweiler et al retrospectively studied 127 infants admitted to intensive care for RSV bronchiolitis and found that 25 (44%) of the 57 ventilated and endotracheally sampled infants had "concomitant bacteria pneumonia" (Duttweiler, Nadal and Frey 2004). Similarly, the retrospective study of Kneyber et al (82 PICU admissions with 65 or 79% ventilated) found that 9 (33%) of the 24 children on whom admission endotracheal aspirates were performed had a positive bacterial culture (Kneyber et al. 2005). Randolph et al retrospectively examined 165 previously healthy infants admitted to the intensive care unit over a 12-year period with laboratory-confirmed RSV infection (63 or 38% required mechanical ventilation). They found that 17.5% – 38% of the 63 intubated infants had "probable" or "possible" bacterial pneumonia (Randolph, Reder and Englund 2004). The incidence of bacterial

pulmonary infection in these retrospective PICU reports is in keeping with that of this prospective study in which all bronchiolitic admissions were included.

Fifty-one percent of the patients with bacteria in their airways and 40% of the RSV-only children had co-morbidities - congenital heart disease, chronic lung disease, large airway abnormality, immunodeficiency, neuromuscular disease. This is in keeping with well-recognised risk factors associated with more severe RSV disease (MacDonald et al. 1982; Langley et al. 1997; Hall 2001; Bloomfield et al. 2004; Thorburn et al. 2004). Co-morbidities did not account for differences in length of ventilation amongst the study groups, but did contribute towards mortality. The high percentage with co-morbidities is most probably also influenced by our centre being the regional paediatric cardiac referral centre, thereby resulting in children with congenital heart disease and bronchiolitis being more likely to be referred to our PICU for intensive care management.

There were fewer deaths in the bacteria-positive group than in those with RSV only. However, when adjusted for those children who had recovered from their RSV infection only to demise later from RSV-unrelated causes, both groups had similar mortalities (2.9% vs. 3.2%). Paediatric Index of Mortality (PIM) is a point of first contact score that is used to assess the risk of death while in the PICU (Shann et al. 1997). The PIM scores for all the groups were similar, suggesting that all groups had matching severity of illness on admission to the PICU. Yet those with positive bacterial cultures required ventilatory support for longer than those with RSV only. Kneyber et al found likewise (Kneyber et al. 2005). Although length of ventilation was significantly different between the groups, other respiratory support and inflammation indices did not differentiate between them (Table 6.1). Perhaps the general inflammatory response once triggered by RSV is not so refined as to be further enhanced by concomitant bacterial infection. Others have also found inflammatory markers unhelpful in differentiating bacterial infection in this group of patients (Toikka et al. 2000; Resch, Gusenleitner and Müller 2003; Kneyber et al 2005). Unfortunately, in

this study no early clinical measurements which would identify those RSV patients with bacterial co-infection could be identified.

Receipt of and length of time on prior antibiotics did not predispose to bacterial co-infection. Moreover, many of the RSV children had had only a day or less (often a single dose close to intubation) of antibiotics. The fact that nearly all the RSV positive children received antibiotics in our PICU limited any interpretation on the impact of antibiotics on their outcome. All those patients with positive bacteriology in their endotracheal secretions had the same organisms isolated on admission surveillance swabs, indicating primary endogenous infection (van Saene, Stoutenbeek and Torres 1992). This reinforces the pathogenesis of lower airways infections in that potential pathogens are carried first in the nasopharynx and then there is migration down the trachea into the lower airways (van Uffelen, van Saene and Lowenberg 1984; van Saene, Stoutenbeek and Torres 1992). The organisms isolated on admission were generally normal community organisms because the majority of the patients were in good health prior to RSV infection and PICU admission (A'Court et al. 1993; Friis et al. 1990). In contrast, *Pseudomonas aeruginosa* was the most common of the abnormal bacteria (Table 6.2). All these patients were carriers of abnormal organisms in their throats, and in most the common denominator for their abnormal carriage was chronic illness (Gilman et al. 1982; van Saene, Stoutenbeek and Torres 1992). Interestingly, *Streptococcus pneumoniae* was isolated from relatively few patients. This could be the result of prior antibiotic use (Sirvent et al. 1997).

Although the majority of LRTI in children are viral in aetiology, mixed viral-bacterial infections are seen in up to a quarter of hospitalized children (Korppi 2003; Michelow et al. 2004). Additionally there is the risk of developing bacterial superinfection with viral LTRI (Hament et al. 1999). These issues have played a role in the World Health Organization's recommendations for the treatment of community-acquired pneumonia to include empirical antibiotics (Korppi 2003; World Health Organisation 2005). Concerns that using antibiotics (in our PICU's case cefotaxime) pre-emptively in this group of

critically ill children would breed antibiotic resistance have been shown to be unfounded in a four-year study (Sarginson et al. 2004). The assessment of the influence of antibiotics on children with severe bronchiolitis would require a prospective randomized control trial.

6.5 *Conclusions*

Up to 40% of patients admitted with severe RSV bronchiolitis were infected with bacteria in their lower airways.

Co-morbidity, namely congenital heart disease, chronic lung disease, large airway abnormality, immunodeficiency, neuromuscular disease, predispose to more severe RSV disease.

7 Mortality rate in children admitted to PICU with RSV infection.

7.1 *Introduction*

World-wide each year 600,000 deaths occur that are directly or indirectly attributable to respiratory syncytial virus (RSV) (Howard et al. 2000). It has been estimated that in children under one year of age nearly 50 deaths per year in the UK (Fleming, Pannell and Cross 2005) and over 200 deaths in the USA (Thompson et al. 2003) are attributable to RSV. RSV has been found to be the most common viral cause of death in children below 5 years of age and especially those less than 1 year old (Thompson et al. 2003). The estimated paediatric all-cause RSV mortality rates were 8.4 (UK) and 5.4 (USA) per 100 000 population in the less than one year old age group, and decreased substantially with increasing age (Thompson et al. 2003; Fleming, Pannell and Cross 2005). However, when restricted to respiratory causes only the UK rate fell to 2.9 deaths per 100 000 population in the less than one year old age group (Fleming, Pannell and Cross 2005). Epidemiological studies have demonstrated that the bronchiolitis-related mortality rate has remained low and stable in the USA, and has declined in the UK over the past two decades (Holman et al. 2003; Panickar et al. 2005).

It is recognised that a considerable proportion of children who die from bronchiolitis have serious underlying conditions / co-morbidity, such as cardiac and central nervous system anomalies, chromosomal abnormalities and immunodeficiencies (Holman et al. 2003; Panickar et al. 2005). However, these pre-existing conditions are often not effectively identified as contributing factors in epidemiological studies which rely on national statistics coding (Shay et al. 1999; Shay et al. 2001; Holman et al. 2003; Thompson et al. 2003; Fleming, Pannell and Cross 2005; Panickar et al. 2005; Jansen et al. 2007).

The reported mortality rate for those hospitalised may be as low as 1 – 3%, but the mortality rate increases in those with severe bronchiolitis requiring

intensive care management (MacDonald et al. 1982; Navas et al. 1992; Langley et al. 1997; Thorburn et al. 2004).

In order to determine the mortality rate and risk factors for death in children with severe RSV infection, a study of all children admitted for paediatric intensive care over an eight year period (i.e. 8 RSV seasons) was performed.

7.2 *Patients ans Methods*

7.2.1 Setting:

20-bed regional multidisciplinary tertiary paediatric intensive care unit (PICU) in university-affiliated children's hospital.

7.2.2 Objectives:

1. To determine the mortality rate in children with severe RSV infection.
2. To determine the risk factors for death in children with severe RSV infection.

7.2.3 Patients:

All children with RSV bronchiolitis, confirmed on RSV antigen testing, immunofluorescence and/or culture that required PICU admission between June 1999 and May 2007 (i.e. encompassing eight consecutive RSV seasons – October to March) were studied.

The data were collected prospectively during the last six RSV / winter seasons (October 2001 onwards) and retrospectively for the first two seasons. PICU admission was utilised as the marker of severity of disease. The study was approved by the Royal Liverpool Children's Hospital's Clinical Audit Department - a subgroup of the Research and Clinical Development Directorate.

7.2.4 Respiratory support:

It is national policy that all children who require intensive care and mechanical respiratory support are transferred to the regional PICU. All forms of mechanical ventilation (conventional and high frequency) and continuous positive airways pressure (CPAP) support are carried out on the PICU. Muscle relaxants are not routinely used. Non-invasive CPAP support is usually performed on our 14-bed high dependency unit (HDU) which is separate from the PICU. More recently some of the regional secondary-level hospitals have been performing non-invasive CPAP support within their paediatric HDU/wards.

Children requiring extracorporeal life support (ECLS) / extracorporeal membrane oxygenation (ECMO) are transferred to one of four national centres.

7.2.5 Microbiologic sampling:

Diagnostic samples of nasopharyngeal aspirates (for RSV detection) and lower airway secretions (for bacterial culture and viral detection) were taken on admission to PICU and thereafter when clinically indicated. From January 2002 onwards RSV surveillance samples were taken on all children during the RSV season (October – March) on admission and twice a week while on PICU. Samples were collected by specialist respiratory physiotherapists or PICU staff members. Additionally see 2.3.1.

7.2.6 Measurements:

7.2.6.1 Virology:

Nasopharyngeal aspirates were tested by the Directigen^{TM} RSV test [Becton Dickinson microbiology systems, Maryland, USA] (1999 – 2004) or NOW^{TM} RSV test [Binax Inc., Maine, USA] (2005 – 2009). These are *in vitro* immunochromatographic assays for the rapid and qualitative detection of RSV antigen directly from nasopharyngeal specimens. Additionally see 2.3.2.1. Samples negative for RSV using the rapid antigen tests were cultured using

Rmix shell vial culture techniques at the Public Health Laboratory (Aintree, Liverpool, UK).

Immunofluorescence for RSV was performed using SimulFluor™ reagents (Chemicon/Millipore, Massachusetts, USA) at the Public Health Laboratory.

7.2.6.2 Bacterial/Yeast:

Diagnostic or clinical samples were processed immediately in a qualitative and semi-quantitative way using standard microbiological methods. Standard methods for identification, typing, and sensitivity patterns were used for all micro-organisms (Brook 1979). Additionally see 2.3.2.2.

7.2.7 Antimicrobial policy:

The empiric use of antibiotics in children with severe bronchiolitis was at the discretion of the attending consultant. Patients with signs of infection received intravenous cefotaxime (50 mg/kg/dose 4 times daily) as first line therapy for 48 hours whilst awaiting culture results. Clinical status on presentation governed whether supplementary intravenous cover with an aminoglycoside, gentamicin (7 mg/kg/day - single daily dose) was added. Antibiotics were rationalised or stopped once culture and sensitivity results became available.

Ribavirin therapy is only considered in immunocompromised children.

7.2.8 Definitions:

Nosocomial RSV infection – when a child admitted to the PICU was RSV negative or from whom no samples were taken as they did not exhibit signs of bronchiolitis, and who then became RSV positive five or more days after the admission (Hall et al. 1978; Raymond and Aujard 2000; Thorburn et al. 2004).

RSV-attributable deaths – Deaths were considered directly attributable to RSV if the patients were still RSV positive when they died or when the RSV infection contributed to the adverse clinical course leading to death (Simon et al. 2008; von Renesse et al. 2009).

7.2.9 Analytic Methods:

Results were expressed as a percentage of the total study population; median and inter-quartile ranges (IQR) were used to describe the demographic distributions. Prediction of mortality using paediatric index of mortality (PIM) was obtained on the patient's first contact with the PICU team (Shann et al. 1997). Standardised mortality ratio (SMR) is the ratio of actual deaths compared to the number predicted (in this study derived from PIM score). Continuous data were analysed using the Wilcoxon-Mann-Whitney (W-M-W) test. Categorical data were analysed using Fisher's exact or McNemar's test. Multivariate analysis was performed using linear and logistic regression analysis.

Statistical calculations were performed with the Statistical Program for Social Science release 11.0.0 (SPSS 11, Chicago, IL). A p value < 0.05 was considered statistically significant. Relative Risk (RR) with 95% confidence intervals (95% CI) calculations was performed with Confidence Interval Analysis software (CIA 2.1.2) from Statistics with Confidence, editors: DG Altman, D Machin, TN Bryant, MJ Gardner; BMJ Books, London (2000).

7.3 *Results*

During the study period 406 RSV-positive patients were admitted to PICU. The patient numbers and deaths for each RSV season are shown on Table 7.1. The causes of death of the 35 RSV-positive children that died are shown in Table 7.2. Overall PICU RSV mortality was 8.6% with a standardised mortality ratio (SMR) of 0.76. During the study period 2009 RSV-positive patients were admitted to the children's hospital, giving a hospital RSV mortality rate of 1.7%. Of the deaths 18 were directly RSV-attributable as the patients were still RSV positive when they died. The remaining 17 children died from non-pneumonitis causes after becoming RSV negative on antigen testing and viral culture, giving a PICU RSV-attributable mortality of 4.4% and a hospital RSV-attributable mortality of 0.9%. RSV infection did not appear on the death certificate in 13 of the 35 deaths (37%).

Table 7.1: RSV-positive children admitted to PICU: 1999 – 2007.

RSV season	Number	Deaths (%)
1999 - 2000	28	1 (4%)
2000 - 2001	39	7 (18%)
2001 - 2002	54	8 (15%)
2002 - 2003	50	3 (6%)
2003 - 2004	94	9 (10%)
2004 - 2005	55	4 (7%)
2005 - 2006	37	3 (8%)
2006 - 2007	49	0 (0%)

Table 7.2: Causes of Deaths in patients with severe RSV infection: (n = 35).

RSV Pneumonitis*	5
Acute RSV bronchiolitis worsening the underlying cardiac condition*	5
Acute Respiratory failure on a background of Chronic Lung Disease*	4
Died on Extracorporeal life support / ECMO* (CHD 3; underlying lung disease 1)	4
Sepsis†	6
Withdrawal† (multiple anomalies 2; severe complex CHD 1; severe myopathy 1)	5
Heart failure† (complex CHD 2)	2
Cardiorespiratory Arrest† (complex CHD + severe tracheomalacia 2)	2
Hypoxic Ischaemic Encephalopathy†	1
Chest infection on underlying Chronic Lung Disease†	1

* deaths while RSV positive

† later deaths once RSV negative

CHD = congenital heart disease

ECMO = extracorporeal membrane oxygenation

Patient demographics of those admitted to PICU are shown in Table 7.3. Mechanical ventilation was required in 96.5% of the children and non-invasive respiratory support only in 3.5%. Clinical characteristics of in the children admitted to PICU are shown in Table 7.4. Analysis of oxygen and ventilation indices (Peters et al. 1998), even though measured, was not undertaken as the high incidences of pre-existing cardiac anomalies and chronic lung disease in both groups would confound interpretation. Pre-existing diseases (chromosomal abnormalities, cardiac lesions, neuromuscular disorders, chronic lung disease, large airway abnormality, immunodeficiency) was present in 187 children (46%) on admission to PICU. Of these children with pre-existing disease, 142 (76%) were admitted primarily for RSV disease and the remainder were post-operative (cardiac and general surgery), neurology, chronic respiratory, or sepsis.

Table 7.3: Patient demographics for children admitted to PICU with severe RSV infection: 1999 – 2007.

	Deaths (n = 35)		**Survivors** (n = 371)		
	Median	**IQR**	**Median**	**IQR**	**p-value***
Age (months)	**5.1**	[2.4 - 13.6]	**2.5**	[1.2 - 9]	< 0.05
Paediatric Index of Mortality[17]	**0.13**	[0.07 - 0.31]	**0.09**	[0.04 - 0.14]	< 0.05
Length of PICU stay (days)	**16.5**	[8 - 31]	**5**	[4 - 9]	< 0.05
Days RSV positive	**7**	[3 - 11]	**3**	[2 - 5]	< 0.05

* Wilcoxon-Mann-Whitney test

All of the RSV deaths had pre-existing medical conditions / co-morbidity – chromosomal abnormalities 29%, cardiac lesions 27%, neuromuscular 15%, chronic lung disease 12%, large airway abnormality 9%, immunodeficiency 9%. Nineteen children (56%) had pre-existing disease in two or more organ systems and carried a significantly higher risk of death (Table 7.4).

There was an interaction effect between pre-existing disease, nosocomial / hospital-acquired RSV infection and mortality (McNemar's test $p < 0.001$). Children with pre-existing disease and nosocomial / acquired RSV infection stayed in PICU longer (W-M-W test $p < 0.001$) and were older ($p < 0.05$). Pre-existing disease and nosocomial / hospital-acquired RSV infection were predictive of death by uni- and multivariate analysis (all p-values < 0.05), but age, gender, previous prematurity, bacterial co-infection and inter-hospital transfer did not add to their prognostic value. Children that died while RSV-positive and those that died later once RSV-negative were similar in age, age group, pre-existing disease (including cardiac and multiple organ system involvement), gender, bacterial co-infection, previous prematurity and inter-hospital transfer (Fisher's exact test all p-values > 0.3). However, the latter group had a longer length of PICU stay - median [IQR]: 31 days [22 - 58] vs. 11 days [8 - 17] (W-M-W test $p < 0.05$).

Data on the prior receipt of prophylactic monoclonal RSV antibody therapy has not been reported as it was only introduced into clinical practice late in the study period and therefore any impact would be difficult to ascertain at this stage. Three children received ribavirin (all were immunocompromised following chemotherapy). Surfactant therapy was utilised in two children with severe pneumonitis.

Five children received ECLS/ECMO, of which the four that died all had pre-existing disease, but not the survivor. One child that died on ECLS/ECMO had RSV and pertussis dual infection.

Table 7.4: Clinical characteristics in children admitted to PICU with severe RSV infection: 1999 – 2007.

	Deaths (n = 35)	**Survivors** (n = 371)		
			RR	**[95% CI]**
Pre-existing disease	**100%**	**41%**	**2.36**	[2.02 - 2.76]
2 or more pre-existing diseases	**54%**	**12%**	**4.38**	[2.92 - 6.58]
Cardiac anomaly	**68%**	**23%**	**2.98**	[2.16 - 4.12]
HPBF cardiac	**65%**	**64%**	**1.01**	[0.71 – 1.44]
Nosocomial infection	**18%**	**6%**	**2.89**	[1.26 – 6.6]
Ex-Pretermer	**9%**	**19%**	**0.46**	[0.15 - 1.39]
Under 6 weeks old	**17%**	**35%**	**0.48**	[0.23 - 1.02]
Under 1 year of age	**71%**	**78%**	**0.91**	[0.72 - 1.13]
Gender (male)	**56%**	**55%**	**1.02**	[0.75 - 1.4]
Bacterial co-infection	**24%**	**36%**	**0.66**	[0.35 - 1.24]
Inter-hospital transfer	**32%**	**52%**	**0.61**	[0.37 - 1.01]

RR = Relative Risk (Death vs. Survival) 95% CI = 95% Confidence Intervals

Pre-existing disease = chromosomal abnormalities, cardiac lesions, neuromuscular disorders, chronic lung disease, large airway abnormality, immunodeficiency.

HPBF cardiac = cardiac lesions with high pulmonary blood flow (e.g. septal and atrioventricular canal defects)

7.4 *Discussion*

This observational cohort study over 8 consecutive RSV seasons found that only children that had pre-existing disease / co-morbidity died from severe RSV infection, i.e. prior good health protected against death. PICU admission was utilised as a marker of severe RSV disease in this study which differs from many other studies that defined hospitalisation as reflecting severe RSV disease (Navas et al. 1992; Langley et al. 1997; Howard et al. 2000; Buckingham et al. 2001; Holman et al. 2003; Purcell and Fergie 2004). Having focused on a higher risk group than many of the previous reports, this study uniquely compared the risk factors in those that died with the survivors within this subgroup with severe RSV disease.

Even though this hospital-based data does not necessarily reflect incidences in the general community which are better reflected in epidemiological / population-based studies, it does reflect risk factors pertinent to RSV infection in the general paediatric population. The assessments and estimates from epidemiological studies are derived from data obtained from diagnosis-based coding categories retrieved from National Surveillance organizations (e.g. Center for Disease Control and Prevention in USA and Health Protection Agency in UK). They therefore suffer from the acknowledged limitation of reliance on specific codes (e.g. for respiratory, circulation deaths) which may not cover all RSV-associated pathologies (Shay et al. 1999; Shay et al. 2001; Holman et al. 2003; Thompson et al. 2003; Fleming, Pannell and Cross 2005; Panickar et al. 2005; Jansen et al. 2007). This would have been the case in a number of the deaths in our study population as their deaths occurred after becoming RSV negative and were not necessarily directly RSV-related/attributable (Table 7.1). The detrimental role or destabilising effect that the RSV infection played in the clinical course of these cases is difficult to assess accurately as the cause of death was often extra-pulmonary. Certainly death certificates did not report the recent RSV infection in nearly 40% of the deaths and this failure would contribute to the RSV influence being missed in epidemiological studies. This may have been a factor in the conclusions of Shay et al. that the majority of RSV-attributable deaths did not occur in

children at high risk for severe RSV disease which is contrary to our data (Shay et al. 2001). Their USA study differed in that the study period was earlier (1979 – 1997) and its epidemiological design relied on cause-specific International Classification of Deaths 9th revision (ICD-9) codes on death certificates. Nearly all the children in the present study that died had well-recognised risk factors for severe RSV disease (MacDonald et al. 1982; Navas et al. 1992; Langley et al. 1997; Handforth, Friedland and Sharland 2000; Buckingham et al. 2001; Purcell and Fergie 2004; Lopez et al. 2007).

In our institution RSV deaths in PICU reflect the total hospital deaths from RSV as it is highly unlikely that any children with severe RSV disease would not be admitted to PICU and more than 85% of hospital deaths occur in PICU. The hospital RSV mortality rate is similar to other studies (Navas et al. 1992; Langley et al. 1997; Handforth, Friedland and Sharland 2000; Buckingham et al. 2001; Holman et al. 2003; Purcell and Fergie 2004; Smyth and Openshaw 2006), even taking into account that our children's hospital is a regional referral centre, thereby collecting cases with severe illness. Additionally, our centre is the regional paediatric cardiac referral centre, resulting in children with congenital heart disease and bronchiolitis being more likely to be referred to our PICU for intensive care management. It is therefore not surprising that one third of deaths in this RSV patient group were in children that were retrieved / transferred from other base hospitals. As found in other studies the number of deaths from RSV each year varied widely (Shay et al. 2001; Thompson et al. 2003; Fleming, Pannell and Cross 2005). Typing of the RSV strain is not performed in our institution, so we are unable to speculate as to whether the strain influenced mortality overall or season on season.

The most significant risk factor for death was pre-existing disease, of which a cardiac anomaly held a particularly high risk. Interestingly, contrary to what one might have presumed, cardiac lesions with high pulmonary blood flows (e.g. endocardial cushion defects, ventriculoseptal defects, truncus arteriosus, etc.) did not stand out from the rest as predisposing to mortality. Congenital heart disease being a significant risk factor for severe disease and death is in

keeping with previous reports (MacDonald et al. 1982; Navas et al. 1992; Handforth, Friedland and Sharland 2000; Buckingham et al. 2001; Holman et al. 2003; Panickar et al. 2005). Earlier studies have identified previous prematurity as a risk factor for hospital admission and death (Hall 1977; Navas et al. 1992; Buckingham et al. 2001; Holman et al. 2003, Purcell and Fergie 2004; Lopez et al. 2007; Simon et al. 2007). However in our study population, when compared to the survivors with severe RSV disease, it was not an additional risk factor for death. Perhaps in this patient group previous prematurity predisposes to severe disease (morbidity), whereas other pre-existing conditions govern mortality. Having said this, caution should be exercised as the sample size may underpower the confidence of interpretations. The pre-existing disease most probably has bearing on the finding that, as a group, those that died were older and stayed in PICU longer.

Pre-existing disease may confound the relationship between nosocomial RSV infection and death. Children with more complex underlying conditions stay in hospital and on PICU longer and thereby have protracted exposure to potential cross infection. This is borne out by the longer length of stay in the group of RSV deaths with its high incidence of both pre-existing disease and nosocomial RSV infection. Previous studies have described an increased incidence of nosocomial RSV infection in children with congenital heart disease, chronic lung disease and immunodeficiency (Hall, Douglas and Geiman 1975; Hall et al. 1986; Buckingham et al. 2001; Purcell and Fergie 2004; Thorburn et al. 2004; Smyth and Openshaw 2006). It highlights not only their vulnerability to nosocomial infection, but also its potential devastating impact on these children. Perhaps this subgroup of patients should be isolated (in a cubicle) and reverse barrier-nursed during the RSV season to minimise nosocomial infection, acknowledging that it may further stretch already strained isolation spaces.

The prolonged days of RSV positivity in the group of RSV deaths may also relate to the high incidence of pre-existing diseases. It has been recognised that patients with underlying conditions may excrete virus / RSV for longer

periods (Hall et al. 1986; Hall 2001; Thorburn et al 2004). So, in addition to susceptibility to nosocomial infection, once infected they constitute a prolonged source for potential cross infection within the unit. RSV prophylaxis (expensive monoclonal antibody therapy) or an eagerly-awaited vaccine (still under development) may offer some protection from RSV infection in the community for this vulnerable group of children with pre-existing disease.

7.5 *Conclusions*

Pre-existing disease / co-morbidity, in particular multiple pre-existing diseases and cardiac anomaly, is associated with a significantly higher risk of death from severe RSV infection.

Nosocomial / hospital-acquired RSV infection is an additional major risk factor for death in children with severe RSV infection.

8 Nosocomial (acquired) RSV infection in ventilated critically ill children – a ten year perspective.

8.1 *Introduction*

Respiratory syncytial virus (RSV) infection of the lower respiratory tract is one of the commonest causes of respiratory failure in children throughout the world. Annually RSV-related hospitalisations account for 20 000 admissions in the UK, 26 000 in Germany, and 90 000 in the USA (Handforth, Friedland and Sharland 2000; Deshpande et al. 2003; Simon et al. 2008). RSV infection is not innocuous with an estimated 600 000 deaths worldwide directly or indirectly attributable to RSV each year (Howard et al. 2000).

RSV epidemics occur at predictable annual intervals during the winter months in moderate climates (Hall 2001). During this time the hospital admissions for RSV infection are greatly increased and cross-infection (nosocomial infection) of inpatients occur. RSV infection accounts for nearly 10% of all admissions to paediatric intensive care units throughout the UK. During the winter patients with RSV may constitute the largest patient group on PICU's around the country (O'Donnell and Darowski 2006). RSV is a highly contagious virus and the mode of nosocomial spread is by close or direct contact with large droplets (fomites) of infected secretions or body fluids (Hall et al. 1981; Hall and Douglas 1981; Hall 2000; Hall 2001). Following direct inoculation of the nasopharynx or eyes, the incubation period is usually 2 - 5 days (Hall and Douglas 1981; Simoes 1999; Hall 2001). Nosocomial infections are a significant complication of hospital care in children and impact on morbidity, costs and mortality (EPINE Working Group 1992; Macartney et al 2000; Raymond and Aujard 2000). 5.5 – 72% of inpatients with RSV infection were found to have acquired their RSV infection in hospital. Most reports relate to children in wards or babies in neonatal units (Hall et al. 1978; Isaacs et al. 1991; Madge et al. 1992; Doherty et al. 1998; Karanfil et al. 1999; Mlinargic-

Galinovic and Varda-Brkic 2000; Cox, Roa and Brandon-Cox 2001; Simon et al. 2008).

Comorbidities/underlying conditions, including congenital heart disease, chronic lung disease, chromosomal anomalies, airways abnormalities, immunodeficiency, neuromuscular impairment – are well recognised risk factors for hospitalisation and severe disease in RSV infection (Buchingham et al. 2001; Hall 2001; Holman et al. 2003; Panickar et al. 2005; Smyth and Openshaw 2006; Jansen et al. 2007). Due to the potential influence that underlying conditions may play comorbidity scores are often utilised to adjust for case-mix when studying nosocomial infections. In adult study populations comorbidity indices / scores such as the Charlson score, the Karofsky index, and the McCabe and Jackson classification have been shown to correlate with risk of nosocomial infections in prevalence surveys (Sax and Pittet 2002). The Charlson score has been adapted for use in a general paediatric population (Muhlemen et al. 2004).

The incidence of nosocomial RSV infection in our regional tertiary referral PICU over a ten year period was investigated, and its impact on morbidity and mortality studied.

8.2 *Patients and Methods*

8.2.1 Setting:

Observational cohort study in a 20-bed regional multidisciplinary tertiary PICU, which includes 8 isolation cubicles, in university-affiliated children's hospital with an annual PICU admission rate of 1100 children.

8.2.2 Objectives:

1. To determine the incidence of nosocomial (PICU-acquired and hospital-acquired) RSV infection in PICU over a ten-year period
2. To determine the impact of nosocomial infection with RSV on mortality

3. To determine the impact of nosocomial infection with RSV on morbidity as determined by duration of ventilation and length of PICU stay.

8.2.3 Patients:

All ventilated children with RSV infection, confirmed on RSV antigen testing, immunofluorescence and/or culture, admitted to PICU between June 1999 and May 2009 (i.e. encompassing ten consecutive RSV seasons – October to March) were studied. Data were collected prospectively during the last eight RSV / winter seasons (October 2001 onwards) and retrospectively for the first two seasons with all the same data points being available as for the prospective data. The study was approved by the Clinical Audit Department – a division of the Directorate of Research and Clinical Development at the Royal Liverpool Children's Hospital NHS Trust.

8.2.4 Sampling:

Diagnostic samples of respiratory tract secretions were taken on admission to PICU and thereafter when clinically indicated. From January 2002 onwards RSV surveillance samples were taken on all children during the RSV season (October – March) on admission and twice a week while on PICU. RSV positive children were sampled more regularly to identify when they became negative, as 3 consecutive RSV antigen negative tests would allow 'release' from an isolation cubicle.

8.2.5 Measurements:

8.2.5.1 Laboratory procedures: RSV was identified on respiratory tract samples at the Health Protection Agency Laboratory (Aintree, Liverpool, UK) using Rmix shell vial culture techniques, and immunofluorescence for RSV using SimulFluor™ reagents (Chemicon/Millipore, Massachusetts, USA), or in-house by immunochromatographic assays: Directigen™ RSV test [Becton

Dickinson microbiology systems, Maryland, USA] (1999 – 2004) or NOW™ RSV test [Binax Inc., Maine, USA] (2005 – 2009). Additionally see 2.3.2.1.

8.2.6 Definitions

Community-acquired RSV infection - Children with RSV bronchiolitis confirmed on RSV antigen testing, immunofluorescence or viral culture from respiratory tract secretions on admission to hospital.

Hospital ward-acquired RSV infection - Children who were RSV negative (or from whom no samples were taken as they had no signs of bronchiolitis) on admission to hospital and who then became RSV positive five or more days after admission to hospital (Hall et al. 1978; Raymond and Aujard 2000; Thorburn et al. 2004).

PICU-acquired RSV infection - Children who were RSV negative (or from whom no samples were taken as they had no signs of bronchiolitis) on admission to PICU and who then became RSV positive five or more days after admission to PICU (Hall et al. 1978; Raymond and Aujard 2000; Thorburn et al. 2004).

RSV-attributable deaths – Deaths were considered directly attributable to RSV if the patients were still RSV positive when they died or when the RSV infection contributed to the adverse clinical course leading to death (Simon et al. 2008; von Renesse et al. 2009).

8.2.7 Infection Control Policy

The RSV infection control policy is based on the prevention of transmission via hands of carers and equipment. The six main manoeuvres are isolation, hand hygiene, protective clothing, care of equipment, surveillance, and minimising risk of cross-infection.

1. Isolation – all known or suspected RSV-positive children are nursed in isolation cubicles. 'Release' from an isolation cubicle is allowed following negative RSV antigen tests on 3 consecutive days.
2. Strict hand hygiene – hand washing before and after contact with each patient.
3. Protective clothing – single use gloves when handling blood and body fluids + single use plastic aprons are worn when in the cubicle and thrown out on exit.
4. Equipment – the environment is cleaned daily with a chlorine-release agent. The bed frame is cleaned before arranging clean linen. Mattresses and pillow covers are thoroughly inspected for signs of wear and tear; if damaged they are disposed of and replaced. If there is no damage but is fouled, they are cleaned with detergent solution. The room is disinfected after discharge.
5. Surveillance – RSV ELISA tests (nasopharyngeal aspirates, broncho-alveolar lavages or tracheal aspirates) on admission to the PICU or development of any respiratory symptoms, and twice weekly during the RSV season.
6. Minimise cross-infection – on each shift, an assigned PICU resident will look after the RSV-positive patients and not be allocated any cardiac, chronic lung disease or immunosuppressed patients.

Monoclonal RSV antibody therapy is not utilised to control RSV cross-infection in inpatients in our children's hospital.

8.2.8 Controlling for confounders: Matched Controls and multivariate analysis.

To counter the confounding factor of comorbidity, controls from the community-acquired RSV group were matched for comorbidity with individuals in the nosocomial (PICU-acquired) RSV group. In order to match similar type and number of underlying conditions/comorbidity within the nosocomial and community-acquired RSV patients, a new score based on an adapted version of the Charlson score (Charlson et al. 1987; Sax and Pittet 2002) was created

taking into account established risk factors for severe RSV disease (Table 8.1) (Buchingham et al. 2001; Hall 2001; Holman et al. 2003; Panickar et al. 2005; Smyth and Openshaw 2006; Jansen et al. 2007). The priority for matching was comorbidity index score, with special attention taken to match the major comorbidities (those scoring 2 points) with the same comorbidities in the matched community-acquired RSV individuals. To avoid selection bias all eligible matching community-acquired RSV patients were included.

Table 8.1: Scores of the Comorbidity Index for patients with RSV infection – adapted from the Charlson score (Charlson et al. 1987).

[a] haemodynamically-significant congenital cardiac anomaly

[b] home oxygen requirement

Illness or Condition	**Score**
Congenital Heart Disease [a]	2
Chronic Lung Disease [b]	2
Large Airways Abnormality [c]	2
Immunodeficiency (congenital or acquired)	2
Chromosomal abnormality	2
Neuromuscular condition	2
Ex-pretermer [d]	1
Other pre-existing illness(es) [e]	1
Total	Sum of fulfilled criteria

[c] any anatomical or dynamic abnormality from laryngeal inlet to main bronchi

[d] born before 36 weeks gestation

[e] metabolic, hepatic, gut, renal, asthma

To investigate whether PICU-acquired and hospital ward-acquired RSV infection is independently associated with mortality or morbidity a multivariate analysis which identified independent factors associated with morbidity and mortality in patients ventilated with RSV infection was conducted.

8.2.9 Analytic Methods:

Results were expressed as a percentage of the total study population; median and inter-quartile ranges (IQR) were used to describe the demographic distributions. Prediction of mortality using pediatric index of mortality (PIM) was obtained on the patient's first contact with the PICU team (Shann et al. 1997). Standardized mortality ratio (SMR) is the ratio of actual deaths compared to the number predicted (in this study derived from PIM score). Continuous data was analyzed using the Wilcoxon-Mann-Whitney (W-M-W) test. Categorical data was analysed using Fisher's exact or Chi-square test. For multivariate analysis, simultaneous multiple regression analysis was used.

To investigate an association of mortality and morbidity as reflected in duration of ventilation and stay on PICU to PICU-acquired and hospital ward-acquired RSV infection simultaneous multiple regression analysis entering age, gender, gestational age, history of prematurity, PIM as an indicator of disease severity on admission and co-morbidities like congenital hear disease, chronic lung disease, immunodeficiency, neuromuscular disorders, chromosomal abnormalities and airway abnormalities as independent and mortality, length of stay, length of stay after RSV infection and length of ventilation as dependent variables was performed. Categorical variables were assigned dummy variables in the regression analysis. A t-statistic of >2 or <-2 was considered indicative of a significant predictive power for prediction of the dependent variable.

Statistical calculations were performed with the Statistical Program for Social Science release 13.0 (SPSS 13, Chicago, IL). A p value < 0.05 was considered statistically significant. Relative Risk (RR) with 95% confidence intervals (95% CI) calculations were performed with Confidence Interval Analysis software (CIA 2.1.2) from *Statistics with Confidence*, editors: DG Altman, D Machin, TN Bryant, MJ Gardner; BMJ Books, London (2000).

8.3 *Results*

During the ten-year study period 491 RSV-positive patients were ventilated on PICU. Forty children acquired their RSV infection following admission to PICU (8.1% of all RSV infection on PICU) and 38 (7.8%) following admission to a hospital ward. Of the 491 RSV-positive ventilated patients, 232 (47%) had been transferred into our regional PICU from other hospitals. During the study period there were 2557 RSV-positive patients admitted to our children's hospital. The patient numbers and deaths for both the community-acquired, PICU- and hospital ward-acquired (nosocomial) RSV infection groups for each RSV season are shown on Table 8.2.

Overall PICU RSV mortality was 8.6% with a standardised mortality ratio (SMR) of 0.78. Number of deaths in the nosocomial group that acquired RSV infection in PICU was 10 (25%) (RR 4.3, 95%CI 2.2 – 8.3) and 8 (21%) that acquired RSV infection in a hospital ward prior to PICU admission (RR 3.6, 95%CI 1.8 – 7.5), compared to 24 (6%) in those with community-acquired RSV infection. The SMR for the PICU-acquired group was 2.02, the hospital ward-acquired group 1.48, and community-acquired group 0.56. Ten of the deaths in children with nosocomial (PICU- and hospital ward-acquired) RSV infection were directly related to RSV giving a RSV-attributable mortality of 12.5% in the nosocomial RSV infection group compared to 4% (n = 15) in the community-acquired RSV group (RR 2.8, 95%CI 1.2 – 7.1).

Patient demographics and clinical characteristics of those admitted to PICU are shown in Table 8.3 and Table 8.4.

Mechanical ventilation was required in 95.3% of the children and non-invasive respiratory support in 4.7%.

Length of PICU admission following becoming RSV positive has also been included as total length of PICU admission would be confounded by the time period required to qualify as nosocomial / acquired RSV infection.

Table 8.2: RSV-positive children admitted to PICU: 1999 – 2009.

RSV season	Number	Deaths
1999 – 2000	28	
Community-acquired	25	1
Hospital ward-acquired	1 (4%)*	0
PICU-acquired	2 (7%)*	0
2000 – 2001	39	
Community-acquired	36	7
Hospital ward-acquired	2 (5%)*	0
PICU-acquired	1 (3%)*	0
2001 – 2002	54	
Community-acquired	37	4
Hospital ward-acquired	5 (9%)*	3
PICU-acquired	12 (22%)*	1
2002 – 2003	50	
Community-acquired	37	2
Hospital ward-acquired	6 (12%)*	0
PICU-acquired	7 (14%)*	1
2003 – 2004	94	
Community-acquired	80	6
Hospital ward-acquired	7 (7%)*	2
PICU-acquired	7 (7%)*	1
2004 – 2005	55	
Community-acquired	42	2
Hospital ward-acquired	7 (13%)*	0
PICU-acquired	7 (13%)*	2
2005 – 2006	37	
Community-acquired	35	2
Hospital ward-acquired	1 (3%)*	0
PICU-acquired	1 (3%)*	1
2006 – 2007	49	
Community-acquired	47	0
Hospital ward-acquired	2 (4%)*	0
PICU-acquired	0 (0%)*	0
2007 – 2008	35	
Community-acquired	30	1
Hospital ward-acquired	4 (11%)*	3
PICU-acquired	1 (3%)*	1
2008 – 2009	50	
Community-acquired	45	2
Hospital ward-acquired	3 (6%)*	0
PICU-acquired	2 (4%)*	0

* Percentage (%) of total PICU admission with RSV infection

Table 8.3: Patient demographics for children ventilated on PICU (1999 - 2009) with community-acquired RSV infection, PICU- and hospital ward-acquired RSV infection, and controls matched for comorbidity – Median [IQR].

	Community-acquired (n = 413)	PICU-acquired (n = 40)	Hospital ward-acquired (n = 38)	Controls [a] (n = 103)
Age (months)	2.6 [1.2 - 8.9]	8.1 [1.9 - 27.4] $p < 0.01$ [†]	5.1 [2.2 - 14.2] $p = 0.02$ [†] $p = 0.24$ [*]	6.5 [2.8 - 19.3] $p = 0.48$ [‡]
Paediatric Index of Mortality (21)	0.08 [.04 - .13]	0.07 [.04 - .12] $p = 0.63$ [†]	0.09 [.04 - .16] $p = 0.18$ [†] $p = 0.36$ [*]	0.08 [.04 - .14] $p = 0.56$ [‡]
Comorbidity Index score	1 [0 - 2]	2 [1 - 3] $p < 0.01$ [†]	2 [2 - 4] $p < 0.01$ [†] $p = 0.35$ [*]	Matched for comorbidity
Length of ventilation (days)	5 [3.5 -8]	18 [10 - 38] $p < 0.01$ [†]	7 [5 - 11] $p < 0.01$ [†] $p < 0.01$ [*]	8 [4 - 12] $p < 0.01$ [‡]
Length of PICU stay (days)	5 [4 - 9]	20 [11.5 - 39] $p < 0.01$ [†]	7 [5 - 11.5] $p = 0.02$ [†] $p < 0.01$ [*]	8 [5 - 12] $p < 0.01$ [‡]
Post RSV length of PICU stay [b] (days)	5 [4 - 8.5]	13 [6 - 28] $p < 0.01$ [†]	6 [4 - 11] $p = 0.07$ [†] $p = 0.04$ [*]	7 [5 - 11] $p < 0.01$ [‡]
Days RSV positive	3 [2 - 5]	3 [1 - 4] $p = 0.66$ [†]	3.5 [1 - 5] $p = 0.72$ [†] $p = 0.81$ [*]	3 [1 - 6] $p = 0.79$ [‡]

Community-acquired = RSV positive on admission to PICU

PICU-acquired = RSV infection acquired (nosocomial) after admission to PICU

Hospital ward-acquired = RSV infection acquired (nosocomial) after admission to hospital

[a] Controls from community-acquired RSV group matched for comorbidity to PICU-acquired RSV patients

[†] Wilcoxon-Mann-Whitney test - community-acquired RSV vs. hospital- or PICU-acquired RSV infection

[‡] Wilcoxon-Mann-Whitney test - controls vs. PICU-acquired RSV infection

[*] Wilcoxon-Mann-Whitney test - PICU-acquired RSV infection vs. hospital-acquired RSV

[b] Length of PICU stay after testing positive for RSV

Table 8.4: Clinical characteristics in children ventilated on PICU with community-acquired RSV infection, PICU- and hospital ward-acquired (nosocomial) RSV infection (1999 - 2009).

	Community-acquired (n = 413)	PICU-acquired (n = 40)	Hospital ward-acquired (n = 38)
Death	24 (6%)	10 (25%)	8 (21%)
RSV-attributable death [a]	15 (4%)	5 (13%)	5 (13%)
Gender (male)	226 (55%)	17 (43%)	25 (66%)
Ex-preterm infant [b]	101 (24%)	5 (13%)	10 (26%)
Less than 6 weeks of age	148 (36%)	9 (23%)	6 (16%)
Congenital Heart Disease [c]	86 (16%)	19 (48%)	21 (55%)
Chronic lung disease [d]	37 (9%)	8 (20%)	9 (24%)
Large airway abnormality [e]	23 (6%)	5 (13%)	6 (16%)
Immunodeficiency [f]	9 (2%)	4 (10%)	2 (5%)
Chromosomal abnormality	25 (6%)	3 (8%)	2 (5%)
Neuromuscular impairment	40 (10%)	6 (15%)	4 (10%)
Other pre-existing illness(es) [g]	34 (8%)	11 (28%)	17 (45%)

Community-acquired = RSV positive on admission to PICU

PICU-acquired = RSV infection acquired (nosocomial) after admission to PICU

Hospital ward-acquired = RSV infection acquired (nosocomial) after admission to hospital

[a] still RSV positive at death

[b] born before 36 weeks gestation

[c] haemodynamically-significant congenital cardiac anomaly, e.g. atrioventriculoseptal defect, Tetralogy of Fallot, hypoplastic left ventricle, truncus arteriosus, etc.

[d] home oxygen requirement

[e] any anatomical or dynamic abnormality from laryngeal inlet to main bronchi, e.g. tracheomalacia, bronchomalacia, laryngeal web, etc.

[f] congenital or acquired, e.g. chemotherapy immunosuppression, Di George syndrome, etc.

[g] metabolic, hepatic, gut, renal, asthma

Comparing the first 5 RSV seasons (1999 – 2004) with the second 5 seasons (2004 – 2009), the incidence of PICU-acquired RSV infections decreased - 29/265 (10.9%) vs. 11/226 (4.9%) (Chi-square p = 0.022; RR 2.2, 95%CI 1.1 – 4.4). During these two time periods the age (W-M-W test p=0.17) and comorbidity index scores (p=0.95) were similar. During these two time periods overall RSV-attributable deaths - 28/265 (10.6%) vs. 14/226 (6.2%) (Chi-square p=0.11, RR 1.71, 95%CI 0.92 – 3.16), and PICU-acquired RSV-attributable deaths - 6/265(2.2%) vs. 4/226 (1.7%) (Fisher's exact test p=0.76, RR 1.28 (95% CI 0.37-4.5) were not different. However the duration of ventilation (medians 6.0 vs. 4.5 days, W-M-W p<0.001) and length of PICU stay (medians 6.5 vs. 5.0 days, p=0.002) were significantly decreased.

To counter the confounding interaction between comorbidity and mortality, children with comorbidities in the PICU-acquired RSV group (n=34) were matched with controls from the community-acquired RSV group (n=103) - Table 8.3. Compared to the comorbidity-matched community-acquired RSV children, age (W-M-W test p=0.48) and PIM (p=0.56) were similar, but deaths (10/34 vs. 17/103 in matched controls) were higher (Chi-square test p=0.04), and post-RSV length of stay and duration of ventilation was longer (W-M-W tests p<0.01) in the PICU-acquired RSV group.

The strength and independence of the predictive power of patient characteristics for parameters reflecting morbidity (duration of ventilation and length of stay on PICU) and mortality were analysed using simultaneous multivariate regression analysis (Table 8.5). The strongest predictor for death were congenital heart disease (t-statistic=5.1and immunodeficiency (t-statistic =5.4). The strongest predictor for duration of ventilation and length of stay on PICU were PICU-acquired RSV infection with a t-statistic of 7.6 and 8.0 respectively. When the simultaneous multivariate regression analysis was repeated for length of stay on PICU after first detection of respiratory syncytial virus to allow for influence of nosocomial RSV infection itself and time until nosocomial infection was detected on length of stay significant predictors of this parameter with a t-statistic >2 and p<0.05 were in order of magnitude of t-statistic airway abnormality (t=5.0), PICU-acquired RSV infection (t =4.6), chronic lung disease (t=3.0), and neuromuscular disease (t=2.3) .

Table 8.5: Factors associated with morbidity (duration of ventilation and length of stay on PICU) and mortality in children ventilated on PICU with RSV infection on simultaneous multivariate regression analysis (n=491).

	Duration of ventilation			Length of stay on PICU			Death		
	Beta	t-statistic	p-value	Beta	t-statistic	p-value	Beta	t-statistic	p-value
Age	0.002	0.039	0.969	0.026	0.636	0.52	0.081	-1.89	0.059
Gender (male)	0.018	-0.483	0.629	0.015	-0.39	0.69	0.014	-0.35	0.72
History of prematurity *	0.041	-1.0	0.31	0.045	-1.127	0.26	-0.04	-0.94	0.347
Paediatric Index of Mortality Shann	0.033	0.8	0.39	0.041	1.07	0.28	0.061	1.52	0.128
Hospital-acquired RSV infection	0.025	-0.61	0.54	0.043	9.8-1.0	0.29	0.031	0.73	0.46
PICU-acquired RSV infection	0.337	7.65	<0.01	0.349	8.0	<0.01	0.017	0.37	0.70
Congenital heart disease †	0.133	2.7	<0.01	0.119	2.5	0.01	0.261	5.2	<0.01
Chronic lung disease ‡	0.127	3.0	<0.01	0.134	3.2	<0.01	0.093	2.13	0.03
Large airway abnormality §	0.247	6.1	<0.01	0.237	5.9	<0.01	0.016	0.4	0.70
Immuno-deficiency •	0.082	2.07	0.038	0.068	1.7	0.08	0.223	5.37	<0.01
Chromosomal abnormality	0.031	-0.748	0.45	-0.02	-0.38	0.70	0.096	2.24	0.025
Neuromuscular impairment	0.07	1.73	0.083	0.079	1.95	0.051	0.133	3.1	<0.01
Other co-morbidities **	0.024	0.57	0.56	0.025	0.60	0.54	0.128	2.9	<0.01

* born before 36 weeks gestation

† haemodynamically-significant congenital cardiac anomaly

‡ home oxygen requirement

§ any anatomical or dynamic abnormality from laryngeal inlet to main bronchi

• congenital or acquired immunodeficiency

** metabolic, hepatic, gut, renal, asthma

8.4 *Discussion*

The major findings of this study of nosocomial RSV infection on PICU are that 8% of the children ventilated in our PICU with RSV infection acquired it on the PICU and another 8% had acquired RSV in a hospital ward. These children had a higher mortality rate, stayed longer on PICU once RSV positive, and were more likely to have an underlying condition/comorbidity than those admitted with community-acquired RSV infection.

There is very little literature on nosocomial RSV infection in PICU (Thorburn et al. 2004; Simon et al. 2008; von Renesse et al. 2009). This study differs from epidemiology-based studies in that it investigates a specific subgroup with severe RSV disease (i.e. requiring ventilation). However, the ten-year incidence of PICU-acquired RSV infection (8%) is close to the 11% of all PICU admissions and the 8% hospital ward-acquired incidence close to the 6% of all inpatients with RSV infection reported in a multi-centred German study (1999 – 2005) (Simon et al. 2008; von Renesse et al. 2009), and the 6% of an earlier inpatient multi-centred Canadian study (1994 - 1996) (Langley et al. 1997a).

A quarter of the children that acquired their RSV infection on PICU died. This finding confirms the reported increased mortality in children with nosocomial RSV infection (Langley et al. 1997a; Simon et al. 2008). The 'attributable' RSV mortality (12.5%) in our nosocomial group is similar to that in a recent German study (15%) in mechanically ventilated children (von Renesse et al. 2009). Owing to the detrimental role or destabilising effect that RSV infection plays in the clinical course of these critically ill children it is difficult to differentiate true RSV-attributable deaths from the deaths where it has played little or no part. Multivariate analysis demonstrated that although there was a tendency toward nosocomial RSV infection being associated with death, the major influence was by its association with comorbidity. However, when compared to RSV controls matched for comorbidity, those with nosocomial (PICU-acquired and hospital ward-acquired) RSV infection had significantly increased mortality rates. The most important element therefore in diminishing these deaths is to eradicate nosocomial spread.

It is recognised that underlying chronic conditions / comorbidity can influence both predisposition to nosocomial infections and length of hospital admission (EPINE Working Group 1992; Macartney et al. 2000; Raymond and Aujard 2000; Sax and Pittet 2002; Muhleman et al. 2004). However, in this study, patients with nosocomial RSV infection had a longer duration of mechanical ventilation and length of stay on PICU which was independently significant on multivariate analysis and when compared to a control group matched for comorbidity. This effect could not be explained by increased disease severity on admission because the risk of mortality score (PIM) was not different between groups. The longer duration of mechanical ventilation and length of stay on PICU would suggest that acquiring RSV infection at a time when the children are sicker (i.e. sick enough to be on PICU) has an additive effect on severity of overall disease. This additive effect is supported by the raised SMR in the nosocomial RSV group, suggesting that they fared worse than their admission risk of death would have predicted. Additionally, chronic injury to lung tissues secondary to the prior mechanical ventilation may predispose to a prolonged disease course upon acquisition of RSV infection.

RSV positivity is tracked in our PICU as there are a limited number of isolation cubicles (8 only), so once RSV negative (3 consecutive negative RSV antigen tests) the patients are moved out onto the open PICU floor. It is interesting that although other studies have described prolonged viral shedding in children with nosocomial RSV this was not found in the present study, even with a high level of comorbidity in the nosocomial RSV group and sicker patients (requiring PICU care as compared to general inpatients) than previous studies (Hall, Douglas and Geimen 1975; Hall et al. 1978; Viera et al. 2003; Simon et al. 2008). Perhaps this is because extended shedding of RSV is found in children with immunodeficiency and there were not many immunodeficient patients (15 of 491) in the present study population (Hall, Douglas and Geiman 1975; Hall 1977; Hall et al. 1986; Wendt et al. 1995; Hall 2001; Madhi et al. 2001).

Seeing that the mode of nosocomial spread is by close or direct contact with large droplets (fomites) of infected secretions the thrust of our nosocomial prevention strategies has been meticulous hand hygiene, barrier precautions

and education. This strategy appears to have been effective. Following a nosocomial RSV outbreak in PICU (2001/2002) there was reinforcement of strict infection control measures. This produced a steady improvement in the subsequent RSV seasons (bar a brief relapse) down to consistently low (0 – 4%) nosocomial infection rates (Table 8.2). This decrease in the incidence of nosocomial RSV infection is highlighted when comparing the first 5 RSV seasons with the second half of the study period. Co-morbidity and mortality were similar, but duration of ventilation and length of PICU stay significantly diminished in the second 5 seasons (both p-values<0.01). Taking into account that nosocomial RSV infection was the strongest predictor of these parameters on multivariate analysis, it is not unexpected that a reduction in nosocomial RSV infections had the potential to reduce duration of ventilation and length of PICU stay in this population. These reductions impact on patient health, PICU bed availability and hospital costs. Staffing levels, number of isolation cubicles, and the transmission factor of visiting teams / house staff had been consistent throughout this 10 year period, so this improvement was most likely the product of persistent reinforcement of basic infection control measures, ongoing education and RSV surveillance.

The lack of a comparable group without RSV infection may limit interpretation in that it may be difficult to differentiate whether the clinical impact is specifically related to acquiring RSV infection or a phenomenon of nosocomial infection in general. The significant predictive power of nosocomial RSV infection on duration of PICU stay persisted when only time of stay from first isolation of RSV was included in the calculation. This rules out that an increase in risk of infection with longer duration of prior hospitalisation generated the relationship of prolonged ventilation and PICU stay and nosocomial RSV infection.

Vaccination of at-risk children would be the ideal answer to prevention, but no effective RSV vaccine is presently available (Hall 2001; Smyth and Openshaw 2006). Prophylaxis with humanised monoclonal RSV antibody in high-risk patients has been shown to decrease the risk of subsequent hospitalisation with RSV (PREVENT Study Group 1997; American Academy of Pediatrics

1998; Impact-RSV Study Group 1998; Hall 2001; Smyth and Openshaw 2006). Even though our at-risk patients are already hospitalised, some reports (largely case series) have suggested using monoclonal RSV antibody therapy to combat intra-hospital spread of nosocomial RSV (Cox, Rao and Brandon-Cox 2001; Heerens, Marshall and Bose 2002; Abadesso et al. 2004; Halasa et al. 2005). Seeing that children with significant comorbidity are at greatest risk of death from nosocomial RSV infection, there is a strong argument for treating this group with monoclonal RSV antibody prophylaxis on admission to hospital during the RSV season. Currently this monoclonal RSV antibody strategy has not been utilised in our PICU as our recent rate of nosocomial RSV infection (as shown in this study) has been low.

8.5 *Conclusions*

PICU-acquired RSV infection accounted for 8% of the children ventilated for RSV infection on our PICU over a period of ten winter seasons.

Mortality is increased in nosocomial (both PICU- and hospital ward-acquired) RSV infection, but principally by its association with pre-existing conditions / comorbidity.

Nosocomial (PICU- and hospital ward-acquired) RSV infection was the strongest predictor of duration of ventilation and length of stay in children on PICU.

Persistent reinforcement of basic infection control measures, ongoing education and RSV surveillance lead to a decrease in the rate of PICU-acquired RSV infection.

9 Conclusion

This thesis describes a series of studies into the clinical impact that severe RSV infection has on critically ill children. The aims of this thesis were:

1) To determine the relationship between hepatic transaminase levels and disease severity, and the aetiology of the elevated transaminase levels in children with severe RSV disease;

2) To determine the prevalence of myocardial involvement and its relationship with disease severity in children with severe RSV bronchiolitis;

3) To investigate whether severe RSV bronchiolitis is associated with reduced right ventricular function in infants without congenital heart disease, and to determine whether reduced right ventricular function is associated with myocardial damage, elevated transaminase levels, and disease severity;

4) To determine the incidence of pulmonary bacterial co-infection and its impact on mortality and morbidity in children with severe RSV bronchiolitis;

5) To determine the mortality rate and the risk factors for death in children admitted to PICU with RSV infection;

6) To determine the incidence of nosocomial (acquired) RSV infection in critically ill children, and the impact of nosocomial RSV infection on mortality and morbidity.

9.1 *Summary of conclusions from the studies*

1) Transaminase level elevation was common in children with severe RSV bronchiolitis. There was no evidence that other common viral infections caused this phenomenon. Disease severity, as judged by duration of ventilation, inotrope use and mortality, was greater in children with elevated transaminase levels. Disease severity as judged by duration of ventilation was independent of the presence of CHD. The elevated transaminase levels may have been a reflection of myocardial failure and/or ischaemic hepatitis

secondary to myocardial failure.

2) Myocardial damage or injury is common in infants with structurally normal hearts and severe RSV lung disease. This may help to explain the increased morbidity and mortality associated with RSV bronchiolitis in children with significant congenital heart disease. Myocardial strain or necrosis may cause a heart already under strain from a structural cardiac abnormality to fail.

3) Right ventricular dysfunction is not uncommon in severe RSV disease, but the degree of dysfunction is not related to the levels of respiratory support or biochemical markers of inflammation. Myocardial and hepatocellular damage can occur in infants with severe RSV bronchiolitis with normal right and left ventricular function.

4) Up to 40% of patients admitted with severe RSV bronchiolitis were infected with bacteria in their lower airways. Co-morbidity, namely congenital heart disease, chronic lung disease, large airway abnormality, immunodeficiency, neuromuscular disease, predispose to more severe RSV disease.

5) Pre-existing disease / co-morbidity, in particular multiple pre-existing diseases and cardiac anomaly, is associated with a significantly higher risk of death from severe RSV disease. Prior good health appears to protect against death from severe RSV disease.

6) PICU-acquired (nosocomial) RSV infection accounted for 8% of the children ventilated for RSV infection on our PICU over a period of ten winter seasons. Mortality is increased in nosocomial RSV infection, but principally by its association with pre-existing conditions / comorbidity. Nosocomial RSV infection was the strongest predictor of duration of ventilation and length of stay in children on PICU. Persistent reinforcement of basic infection control measures, ongoing education and RSV surveillance lead to a decrease in the rate of nosocomial RSV infection.

9.2 *Future Research*

9.2.1 RSV Hepatitis?

There was no evidence in my studies that co-infection with other common viral infections resulted in more severe respiratory disease and caused the elevated transaminase levels / hepatitis. The lack of infection with either known hepatitis viruses or respiratory viruses known to cause hepatitis may imply that RSV itself caused the hepatitis. There is a single case report of RSV alone being isolated from liver tissue in a 7-month old infant with bronchiolitis and extrahepatic biliary atresia (Nadal et al. 1990). Conclusive proof of hepatic RSV infection would require a liver biopsy. Understandably this would be inappropriate in live patients on ethical grounds, but following parental consent could be performed on the children that die from (or with) severe RSV infection. Clinical and biochemical data could be correlated with the histology and viral PCR results to assess whether the entity of RSV hepatitis (i.e. RSV infection of hepatocytes) exists.

9.2.2 RSV myocarditis?

Myocardial damage or injury is common in infants with structurally normal hearts and severe RSV lung disease. RSV has been isolated from myocardial tissue in immunodeficient individuals (Fishaut, Tubergen and McIntosh 1980). As with RSV hepatitis, conclusive proof of RSV myocarditis would require sampling the heart muscle. Once again this would be inappropriate in live patients on ethical grounds, but following parental consent could be performed on children that die from (or with) severe RSV infection. Histology and/or viral proof or disproof of RSV myocarditis could be sought.

9.2.3 Additional markers of myocardial damage or strain?

Prior studies (including from this thesis) have reported raised cardiac troponin levels (cTnT) in 35 - 54.5% of children with RSV infection (Checchia et al. 2000; Moynihan et al. 2003, Eisenhut et al. 2004). I found elevations in cTnT to be transient and echocardiographic assessment of left ventricular function

failed to demonstrate evidence of reduction in myocardial contractility. It raises the possibility that using cardiac troponin alone may be overestimating the true incidence of myocardial involvement in children with RSV infection, although the patients presented in the studies in this thesis all have severe RSV infection as reflected by the need for PICU treatment. Perhaps utilising additional biochemical markers of myocardial insufficiency (e.g. BNP, GP-BB, H-FABP) may confirm these findings or establish different patterns of myocardial involvement.

Brain natriuretic peptide or B-type natriuretic peptide (BNP) is a 32 aminoacid polypeptide secreted by the ventricles of the heart in response to excessive stretching of cardiomyocytes. The plasma concentrations of both BNP and NT-proBNP (the amino terminal segment of BNP's prohormone) are increased in patients with asymptomatic or symptomatic left and right ventricular dysfunction. In children with congestive heart failure, BNP and NT-proBNP levels correlate with functional capacity (Kemp et al. 2004; Doust et al. 2005). BNP can differentiate cardiac from pulmonary causes in infants with respiratory distress (Nir and Nasser 2005).

Glycogen phosphorylase (GP) catalyses the breakdown of glycogen in the sarcoplasmic reticulum. One of it's isoenzymes, BB, is found in brain and heart. GP-BB is released from the sarcoplasmic reticulum into the cytoplasm, and then into the circulation through the damaged cell membrane. It has been reported to be a useful marker of myocardial damage (Kemp et al. 2004).

Heart fatty acid binding protein (H-FABP), a 132 aminoacid polypeptide, is released rapidly from the myocardium into the bloodstream after ischaemic injury. It also appears rapidly in urine, and the urinary concentration correlates with the severity of the myocardial injury (Kemp et al. 2004). The presence of these cytosolic proteins in the plasma of patients implies that cell membrane permeability may be compromised in congestive heart failure and myocardial strain (Kemp et al. 2004; Doust et al. 2005; Nir and Nasser 2005).

9.2.4 Myocardial depressant factors?

Myocardial dysfunction is well described in sepsis with negatively inotropic factors, so-called myocardial depressant factors, being proposed as a mechanism. Certain cytokines (e.g. IL-6, TNFα, IL-1ß) have been reported to mediate myocardial depression (Giroir and Stromberg 2000; Pathan et al. 2004). The antiviral and cell-mediated immune reaction to RSV infection is primarily coordinated by RSV-infected respiratory epithelial cells and by alveolar macrophages. The respiratory epithelial cells release cytokines (TNF, interferons, interleukins) and chemokines. These cytokines and chemokines may enter the systemic circulation and impact on myocytes (Openshaw , Culley and Olszewska 2001; Tripp 2004; Openshaw 2005; Tripp, Oshansky and Alvarez 2005). Echocardiographic assessment of myocardial function, measurement of biochemical markers of myocardial damage or strain (e.g. cTnT, BNP, etc.), combined with clinical parameters could be correlated with known 'myocardial depressant' cytokine levels in patients with RSV infection to ascertain whether increased serum 'myocardial depressant' cytokine levels are associated with severe disease and/or myocardial dysfunction. Recent multiplexed DNA micro-array techniques make testing of a number of targeted cytokines easier and less costly.

9.2.5 Right ventricular dysfunction?

Significantly reduced right ventricular function was found in 20% of infants without congenital heart disease ventilated for RSV bronchiolitis. To differentiate whether it is RSV per se that causes right ventricular impairment or severe lung disease secondarily impacting on the ventricular function, a study comparing a control group with non-RSV severe lung disease with a study group with severe RSV bronchiolitis is indicated.

9.2.6 Bacterial co-infection in severe RSV bronchiolitis?

My study over 3 consecutive RSV seasons evaluating bacterial pulmonary co-infection found that 42% of children admitted for severe RSV infection harboured bacterial pathogens in their lower airways and 22% had strong

microbiological evidence of bacterial co-infection. I found inflammatory markers (CRP + WCC) to be unhelpful in differentiating bacterial infection in this group of patient with severe RSV bronchiolitis, as have other investigators (Toikka et al. 2000; Resch, Gusenleitner and Müller 2003; Kneyber et al 2005). Perhaps utilising such a subgroup with diagnosed bacterial co-infection would be an ideal study group to investigate the value/efficacy of reportedly more bacterial-specific inflammatory markers (e.g. procalcitonin, TREM-1/triggering receptor expressed on myeloid cells, IL-6) (Gibot et al 2004; van Rossum, Wulkan,Oudesluys-Murphy 2004).

9.2.7 Influence of antibiotics on children with severe bronchiolitis?

My study over 3 consecutive RSV seasons evaluating bacterial pulmonary co-infection found that receipt of and length of time on prior antibiotics did not predispose to bacterial co-infection. Nearly all the RSV positive children received antibiotics in our PICU, limiting any interpretation on the impact of antibiotics on their outcome. Up to 40% of patients admitted with severe RSV bronchiolitis were infected with bacteria in their lower airways and those with bacterial co-infection were ventilated for longer than those with RSV alone. Perhaps the use of prophylactic antibiotic on admission to PICU may be beneficial in this group of patients with severe RSV bronchiolitis. The assessment of the influence of antibiotics on children with severe bronchiolitis would require a prospective randomized control trial.

9.2.8 Chronic excretion / persistent shedders of RSV?

RSV positivity is tracked in our PICU as there are a limited number of isolation cubicles (8 only), so once RSV negative (3 consecutive negative RSV antigen tests) the patients are moved out onto the open PICU floor. RSV is usually shed for 4 – 6 days, but shedding can range from 3 – 27 days (Hall, Douglas and Geiman 1975; Hall et al. 1976; Hall et al. 1977; Hall et al. 1978). The duration of viral shedding can correlate with severity of illness. Neonates may shed RSV for longer periods of time than older patients. During an outbreak of nosocomial RSV infection on our PICU (2001/2002) a second smaller peak in

nosocomial RSV infections (February 2002) was noted, the source was surmised to be patients who were persistent shedders of RSV. Two chronic PICU patients were identified who had tested negative on consecutive RSV ELISA tests, thereby being allowed out of their isolation cubicle into the 'open' unit, only to once again test RSV ELISA positive later. Quality of sample (i.e. poor quantity of nasal secretions obtained) can influence the sensitivity of RSV ELISA tests. The quality of the RSV ELISA test in detecting low concentrations of virus may also play a role. The ELISA tests are generally 80 – 90% sensitive and specific when compared to immunofluorescence and viral culture (Smyth and Openshaw 2006). In critically ill children, viral shedding can fluctuate (Hall et al. 1977). Both of the suspected persistent shedders had congenital heart disease along with other co-morbidity, and both had been ventilated for more than 3 weeks (i.e. seriously ill children). The suspicion is that this subgroup of RSV-positive patients (chronic with comorbidities) may predispose to subclinical persistent shedding / chronic excretion of the virus. To conclusively establish this hypothesis these persistent shedders would need to firstly be identified and then tracked throughout their entire stay in PICU (and even in hospital). In real terms this would require that all samples on all RSV-positive patients sent for RSV testing be kept so that any patient that subsequently tests positive again can be identified. Once identified, their samples can be typed (to confirm 're-activation' of the same virus) and PCR analysis performed to measure viral loads.

9.3 *Conclusion of thesis*

The studies reported in this thesis have documented marked pulmonary and extrapulmonary clinical implications for children with severe RSV disease. RSV infection is not innocuous and causes significant morbidity and mortality worldwide every year. Not all children are equal when it comes to infection by RSV as those with underlying chronic conditions/comorbidity carry an additional risk of severe disease, nosocomial infection and death.

This thesis has demonstrated that an extrapulmonary (myocardial and hepatocellular in particular) impact from severe RSV bronchiolitis is common. These extrapulmonary effects appear to be indirect, being secondary to parenchymal lung disease and its causative respiratory compromise or consequential of prowling inflammatory mediators, rather than direct RSV effects (i.e. site-specific RSV infection of that tissue). Although host genetic factors manipulate the patient's immune-augmented response, acquired (comorbidity) factors govern and compromise their reserve at both pulmonary and distant extrapulmonary sites.

Investigating the clinical inter-relationship between the pulmonary and extrapulmonary systems and highlighting how they impact on morbidity and mortality improves both the insight into and the management of these children with severe RSV infection.

Although the basic sentiments of Reynolds and Cook in 1963 – "*Oxygen is vitally important in bronchiolitis and there is little evidence that any other treatment is useful*" (Reynolds and Cook 1963) – still ring true, our understanding of RSV disease, its impact on multiple organ systems and its treatment options has progressed over time.

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Appendix

Published papers

1. Hepatitis associated with severe respiratory syncytial virus positive lower respiratory tract infection.

   Eisenhut M, **Thorburn K**

   Scandinavian Journal of Infectious Diseases 2002; 34:235

2. Transaminase levels in ventilated children with respiratory syncytial virus bronchiolitis.

   Eisenhut M, **Thorburn K**, Ahmed T

   Intensive Care Medicine 2004; 30: 931-4

3. Cardiac troponin T levels and myocardial involvement in children with severe respiratory syncytial virus lung disease
   Eisenhut M, Sidaras D, Johnson R, Newland P, **Thorburn K**

   Acta Paediatrica 2004; 93: 887-90.

4. Respiratory syncytial virus outbreak in a pediatric intensive care unit.

   Thorburn K, Kerr S, Taylor N, van Saene HFK
   Journal of Hospital Infections 2004; 57: 194-201

5. The potential impact of prophylaxis against bronchiolitis due to the respiratory syncytial virus in children with congenital cardiac malformations.
   Rackham OJ, **Thorburn K**, Kerr SJ
   Cardiology of the Young 2005; 15: 251-5

6. High incidence of pulmonary bacterial co-infection in children ventilated for severe respiratory syncytial virus (RSV) bronchiolitis
   Thorburn K, Harigopal S, Reddy V, Taylor N, van Saene HKF
   Thorax 2006; 61: 611-615

7. Think outside the box: extrapulmonary manifestations of severe respiratory syncytial virus infection.
   Thorburn K, Hart CA
   Critical Care 2006; 10:159

8. Pulmonary bacterial co-infection in children ventilated for severe respiratory syncytial virus (RSV) bronchiolitis is common.
   Thorburn K, van Saene HFK
   Intensive Care Medicine 2007; 33: 565

9. Bacterial co-infection and the interpretation of immunological data from BAL fluid specimens in severe RSV bronchiolitis
   Thorburn K
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